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**Optimising the Therapeutic Ratio of
Radioimmunotherapy; an Investigation of the
Roles of Chimerisation, Fractionation and
Radiation Dosimetry.**

Dr John Albert Violet

University College London

PhD Oncology

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Dedication

I am deeply grateful to all the staff in the academic department of Oncology at the Royal Free for their help and support. In particular I am deeply grateful to my supervisors who have encouraged and supported me during the last three years.

Many thanks also to all the clinical staff within the departments of Nuclear Medicine, Haematology and Moore ward; without them the running of the clinical trials would not have been possible. Additional thanks also to Dr Steve Marley at the Hammersmith hospital for his time teaching me how to perform haematological stem cell assays. Thanks also to the staff at CRUK who have regulated the CHT-25 and A5B7 studies.

I could not have written this thesis without the help and support of my wife over the last three years.

Finally thanks to my mother, who was diagnosed, treated and died of cancer as I worked on this PhD. Experiencing her loss of life has reminded me of the need to improve the way in which we treat cancer and has inspired me in my work.

Abstract

Radioimmunotherapy (RIT) is a targeted form of treatment for cancer which uses tumour-associated antibodies to selectively deliver a therapeutic radionuclide to sites of disease. In lymphoma, radioimmunotherapy has proved a remarkably effective agent due to the high radiosensitivity of the tumour and its propensity to undergo apoptosis following irradiation. However, success in the treatment of the more radioresistant common solid tumours has been less successful, and for these patients RIT remains investigative. The effectiveness of RIT is limited by non-specific irradiation of normal tissues whilst antibody remains in the circulation, in particular bone marrow, and also by immunogenicity of antibody which does not allow for repeated therapy.

In the first chapter I have hypothesised that lymphomas expressing the interleukin-2 receptor might be effectively treated using a radiolabelled antibody to this receptor. In a phase I/II clinical study, ^{131}I labelled CHT-25, a chimeric antibody against the IL-2R α chain, has shown encouraging evidence of efficacy in the 9 patients with multiply- relapsed lymphomas treated so far. In addition, use of this antibody has been associated with low immunogenicity allowing for repeated therapies to be given.

In the second chapter I have hypothesised that dosimetry led, individual patient therapy, might further optimise ^{131}I CHT-25 treatment. To investigate this I have used marrow toxicity as a biological assay of absorbed dose and shown that simple, but individual, patient biodistribution indices correlate better with observed toxicity than the population-based dose estimates currently employed.

I have proposed that adoption of individual patient dosimetry using tracer studies is worthy of further investigation for the future development of ^{131}I -CHT-25.

In the third chapter I have hypothesised that dose fractionation might improve the therapeutic ratio of RIT. This has been investigated in a pre-clinical human colorectal xenograft model in nude mice using ^{131}I -A5B7, a murine antibody against CEA. In this setting fractionation neither reduces normal tissue toxicity nor increases the effectiveness of therapy.

This thesis demonstrates, using both pre-clinical and clinical data, how the therapeutic ratio of RIT might be improved through antibody design, leading to reduced immunogenicity, dose fractionation and radiation dosimetry, and proposes how these approaches might be used to optimise the effectiveness of RIT in the clinic.

Declaration

All of the work in this thesis has been performed by me, personally, except where due acknowledgement has been made.

John Albert Violet

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Abbreviations

ABMT	Autologous bone marrow transplantation
ADCC	Antibody directed cellular cytotoxicity
ADEPT	Antibody directed enzyme prodrug therapy
AE	Adverse event
AFP	Alpha fetoprotein
ALT	Alanine Amino-transferase
AST	Aspartate Amino-transferase
ARSAC	Administration of Radioactive Substances Committee
AUC	Area under the curve
BP	Blood pressure
BSA	Body surface area
CEA	Carcinoembryonic antigen
CDC	Complement dependent cytotoxicity
CDR	Complementary determining residue
CFU _{GM}	Colony forming unit for granulocytes and macrophages
CFU _S	Colony forming units for the spleen
CFU _F	Colony forming units for fibroblasts
C _H	Constant heavy chain
C _L	Constant light chain
CR	Complete response
CRC	Colorectal cancer
CRF	Case report form
CRUK	Cancer research UK
CTC	Common Toxicity Criteria

CT	Computerised Tomography
DDX	Doctors' and Dentists' exemption
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
EBRT	External beam radiotherapy
ECG	Electro-Cardiogram
ECHO	Echocardiogram
EDTA	Ethlene-Diamine Tetra-Acetic Acid
ELISA	Enzyme linked immunosoebance assay
F _{AB}	Fragment antigen binding
FACS	Fluorescent Activated Cell Sorter
FDG	Fluoro-2-deoxy-D glucose
FDG-PET	Fluoro-2-deoxy-D glucose positron emission tomography
F _C	Crystallisable fragment
FDA	Food and Drug Administration
GCP	Good clinical practice
GGT	Gamma Glutamyl-transferase
HACA	Human anti chimeric antibody
HAHA	Human anti human antibody
HAMA	Human anti mouse antibody
Hb	Haemoglobin
HCG	Human chorionic gonadotrophin
HD	Hodgkin's disease
HLA	Human Leukocyte antigen
HPLC	High performance liquid chromatography

HTLV	Human T-Cell Leukaemia virus
ICH GCP	International Conference on Harmonisation of good clinical practice
IgG	Immunoglobulin (class G)
IL-2R	Interleukin 2 receptor
IL-2R α	Interleukin 2 receptor alpha chain
IL-2R β	Interleukin 2 receptor beta chain
IL-2R γ	Interleukin 2 receptor gamma chain
INR	International Normalised Ratio
IV	Intravenous
kD	Dissociation constant
KeV	kiloelectron volts
L	Litre
LET	Linear energy transfer
LREC	Local Research Ethics Committee
M	Molar
MBq	Mega Becquerel
MBq..s	Megabecquerel.seconds
Mg	milligrams
Mins	minutes
ml	millilitres
MIRD	Medical internal Radiation dose Committee
MR	Minor response
MRI	Magnetic Resonance Imaging
MTD	Maximum tolerated dose

MUGA	Multi Gated acquisition
NHL	Non Hodgkin's lymphoma
PBS	Phosphate buffered saline
PBSCT	Peripheral blood stem cell transplantation
PD	Progressive disease
PR	Partial response
RIGS	Radioimmunoguided surgery
RIT	Radioimmunotherapy
ROI	Regions of interest
RR	Response rate
SAE	Serious adverse event
scFv	Single chain variable fragment
SD	Stable disease
SDR	Specificity determining residue
SPECT	Single photon emission computerised tomography
SUV	Standardised uptake value
$T_{1/2}$	Half-life
T4	Thyroxine
U/S	Ultrasound
V_H	Variable heavy chain
V_L	Variable light chain
VOI	Voxel of interest
WCC	White cell count
WHO	World health organisation
5FU	5-fluorouracil

Introduction

1.1 Antibody Targeted therapies for Cancer

1.1.1 Current approaches for the treatment of Cancer

Cancer is the second largest cause of death in developed countries and causes 25% of all deaths in the United Kingdom (Cancer Research UK, 2002).

Although surgical removal of the primary tumour, often combined with irradiation or chemotherapy, frequently offers successful treatment of truly localised neoplasia, in metastatic and advanced disease, where systemic chemotherapy remains the principle form of treatment, cure is rarely possible.

In adults, cytotoxic chemotherapy became established in the 1970's as a curative treatment in advanced Hodgkin's disease (1), non Hodgkin's lymphoma (2) and teratoma of the testis (3). Its success in these tumour types suggested a potential use as a definitive or as an adjuvant therapy in asymptomatic patients with the common epithelial tumours, with the aim of improving survival. However the successes seen in the haematological malignancies and germ cell tumours have not been achieved in the more common cancers. Despite the refinement of treatment by the use of new drugs, combination regimes and high dose approaches (4-7), established metastatic disease remains almost universally fatal and for these patients the use of cytotoxic chemotherapy remains essentially palliative (8). Moreover even in the adjuvant setting, where the possibility of cure is greatest, an overall survival benefit of less than 5% has been achieved by chemotherapy in the treatment of breast, colon, and head and neck cancers (9-11). In 1986 it was estimated that the contribution of chemotherapy to overall survival was 4.3% (12) and a more recent review from 2004 has suggested a figure of between of 2.1 and 2.3 % (13).

A number of factors are thought to be important contributors to the poor efficacy of systemic chemotherapy. Drug resistance may occur through a number of mechanisms (14;15) and remains a significant barrier to successful therapy (16;17). Another important factor that limits all systemic approaches is their narrow therapeutic ratio. None of the targets of current chemotherapy drugs are cancer specific; DNA and RNA synthesis, microtubule assembly and function (vinca alkaloids and Taxanes) and topoisomerases (etoposide, camptothecins) are all required by normal cells, especially rapidly proliferating normal cells such as those in bone marrow, as well as by cancer cells. As a result, although systemic chemotherapy may be very effective at killing tumour cells, it fails to discriminate between normal and malignant tissues. This failure to discriminate between normal tissues and tumour leads to significant normal tissue toxicity which causes considerable morbidity. Moreover normal tissue toxicity also limits the dose and duration of treatment that can be safely administered leading to reduced efficacy. One strategy to overcome this limitation of systemic chemotherapy is to selectively deliver the cytotoxic agent to tumour cells and so achieve high potency in diseased tissue whilst minimising normal tissue toxicity. Tumour specific antibodies are one delivery vehicle that has been used to deliver targeted therapy.

Radioimmunotherapy (RIT) refers to the use of antibodies to selectively deliver a therapeutic radionuclide to sites of tumour involvement and is the prime focus of this thesis. The effectiveness of RIT is highly dependent upon tumour type; considerable success has been seen in the treatment of B-cell lymphoma and

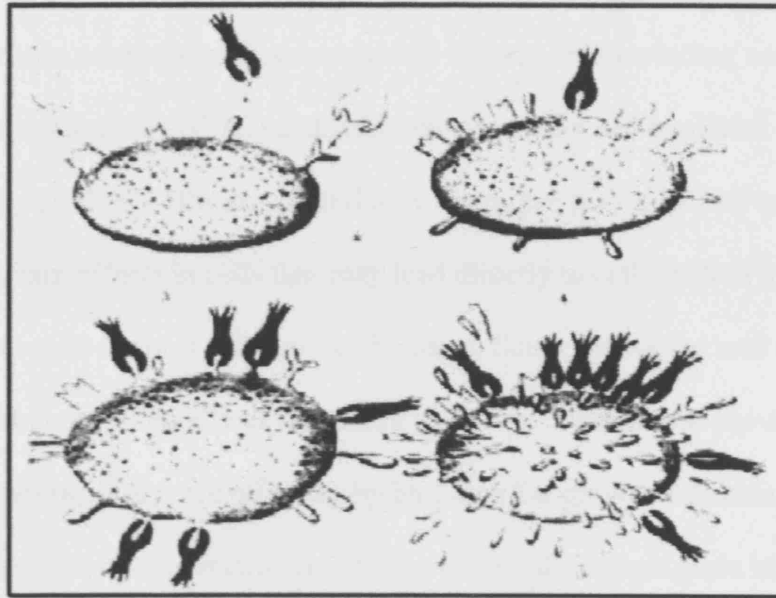
⁹⁰Y-ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals) was the first commercially available radiolabelled antibody to be approved by the American Food and Drug Administration for cancer treatment. The success of RIT in these tumours however is, at least in part, related to properties inherent in their biology and radiobiology. By contrast RIT for the more common epithelial tumours remains essentially investigative and new approaches are required to take therapy into the clinic.

In this work the RIT of T-cell lymphoma and Hodgkin's disease, tumour types with similarities to B-cell NHL in which RIT has already been shown to be effective, has been studied. In addition colorectal cancer, a tumour type typical of the more resistant common epithelial malignancies, has also been investigated.

1.1.2 Immune Targeted Therapies for Cancer

Almost a century ago Paul Ehrlich proposed that by utilising target specific antisera and so exploiting inherent differences between healthy and diseased cells, therapeutic agents could be designed to specifically target diseased cells whilst leaving healthy cells unperturbed.

Figure 1.1: Cellular targeting through lock and key surface proteins and circulating antisera. Diagram from Dr Paul Ehrlich originally from a lecture given in 1890 (18)



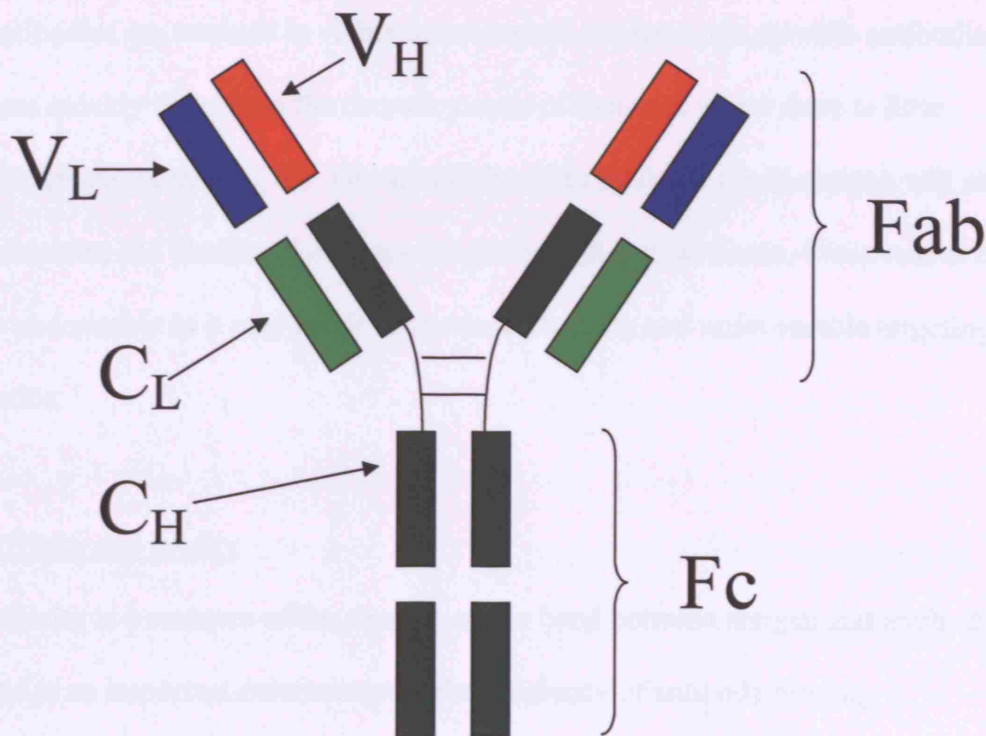
1.1.3 Antibodies as targeting agents

Antibodies have a complex antigen-binding region and can bind with high affinity to the diverse proteins and carbohydrate structures found in malignant cells. Whole antibodies are made up of two identical heavy chains and two identical light chains held together by interchain disulphide bonds. The N-terminal portions of both the heavy and light chains show considerable variability and are responsible for antigen recognition, whereas the remaining parts of the chains are relatively constant and are responsible for recruiting host effector mechanisms. Although antibodies bind to antigen by a number of weak bonds, the overall strength of this binding is high when antigen meets the appropriate antibody because of the close configuration of antigen and antibody binding sites. The configuration recognised on the antigen is called the epitope and several epitopes may be present on large antigens. The typical, naturally

occurring antibody response is directed against several epitopes on one antigen and is referred to as a polyclonal response (19).

In normal physiology antibodies may elicit a number of host mediated defence mechanisms to eradicate pathogens and kill tumour cells including complement-dependent cytotoxicity (CDC) and antibody-dependent cell mediated cytotoxicity (ADCC). Binding of antibody to antigen may also lead to a number of downstream effects in cells that may lead directly to cell death or apoptosis without the need for host effector mechanisms. Some antibodies lead to cell kill by 'switching on' intracellular signalling pathways, for example pro-apoptotic signals, whereas others are effective by blocking the growth enhancing effects of cytokines or by down regulation of growth stimulating antigenic binding sites.

Figure 1.2: Schematic diagram of whole IgG Antibody. This consists of 2 variable light chains (V_L), 2 variable heavy chains (V_H), 2 constant light chains (C_L) and 6 constant heavy chains ($2 \times C_{H1}$, $2 \times C_{H2}$, $2 \times C_{H3}$). The N terminal Fab regions show a high degree of variability and are responsible for antigen binding, whilst the C terminal Fc portions are constant in structure within any immunoglobulin subclass and are responsible for recruiting host defence mechanisms.



The effective targeting of tumours by antibodies is dependent upon a number of factors relating to the antibody (specificity and selectivity, affinity and avidity), properties of the targeted antigen (density, availability, shedding and heterogeneity of expression) and tumour characteristics (vascularity, blood flow and permeability).

1.1.4 Antibody characteristics that influence antibody targeting

Specificity and Selectivity

The specificity of an antibody for its antigen will influence its biodistribution with specific antibodies being retained within tumour areas, whereas non-specific antibodies are not. The tumour to normal tissue ratio of specific antibody steadily increases with time, whereas for non-specific antibody the ratios remain low. Also, microdistribution studies have shown that specific antibodies are retained in viable tumour areas, whereas non-specific antibodies pass quickly through to the necrotic centre of tumours, where there is little therapeutic potential (20). The selectivity of an antibody for its antigen will also determine the likelihood of cross-reactivity with normal tissue. Cross-reactivity is undesirable as it may result in unwanted toxicity and unfavourable targeting ratios.

Affinity and avidity

Affinity is a measure of the strength of the bond between antigen and antibody and is an important determinant of the efficiency of antibody binding.

Measurements of affinity relate to equilibrium conditions, and can be expressed by a kinetic 'on-rate' and 'off rate'. The affinity of an antibody for its antigen is important for targeting, especially when antigen concentration is low within a tumour. High affinity antibodies often have a low 'off rate' (i.e. once they bind antigen they remain bound to it). Theoretically one might expect that the use of high affinity as opposed to low affinity antibodies would result in increased tumour uptake and retention of antibody. Although high affinity antibodies have

been shown to deposit heavily in areas closely adjacent to tumour vasculature they may also demonstrate very little diffusion through the tumour (21). It has been proposed that successful antibody localisation in these areas may lead to an effective 'binding site barrier' which reduces the ability of antibody to penetrate deeper into tumour (22;23). This may be overcome to some degree by increasing the administered amount of antibody or by prolonging the infusion time, but this may result in lower tumour to blood ratios as more antibody is present systemically (24). It has also been suggested that the use of high affinity antibodies could be disadvantageous in the presence of circulating antigen and result in lower levels of tumour binding and localisation than if a lower affinity antibody were utilised (25). Low affinity antibodies on the other hand, with a rapid off rate, diffuse further into the tumour, but are not retained for as long as higher affinity antibodies and therefore do not achieve such selective targeting (26). However since intact antibodies and most antigens are multivalent, the tendency to bind antigen depends upon not just affinity but also the number of binding sites. Avidity therefore describes the overall tendency of antibody to bind to antigen and polyvalent interactions may result in a large improvement in functional affinity. For example, IgG, which is a bivalent molecule, has an approximately 1000-fold increase in functional affinity in comparison to a single F_{ab} fragment (27). It has been demonstrated, using image registration techniques that there is a correlation between radiolabelled antibody tumour distribution and tumour morphology, with bivalent and trivalent antibodies having longer retention in viable tumour areas compared to monovalent antibodies (28;29).

1.1.5 Antigen characteristics that influence tumour targeting

Whilst tumour cells are ultimately derived from normal progenitor cells, transformation to a malignant phenotype is often accompanied by changes in antigenicity. The expression of tumour-associated antigens may arise due to a variety of mechanisms including alterations in glycosylation patterns, expression of virally encoded genes, chromosomal translocations or over expression of cellular oncogenes. Tumour associated antigens are therefore a diverse group, often with poorly defined functions. Important characteristics of a target antigen that will determine its suitability for antibody directed therapy include its tumour specificity, the presence of a soluble form, the antigen's normal function and the distribution of the antigen in the tumour and normal tissues (27;30)

Antigen Specificity

Tumour antigens may be unique to the tumour or be expressed on tumour cells to a higher degree than normal tissue. Antigens that are truly unique to tumour cells are unusual and include immunoglobulin idiotypes in B cell lymphomas. B cell lymphomas and leukaemias represent the abnormal proliferation of a single clone of transformed cells (31). As a result these transformed cells all express the same cell surface immunoglobulin that contains a unique epitope that may be used to differentiate the cancer cell from other normal cells (32). Most other tumour-associated antigens are not tumour specific however and may be expressed to a variable degree on normal tissue. Antibody targeting is still possible with these antigens provided the normal tissue expression is minimal,

occurs in non-essential tissues or if the normal tissues expressing the antigen are inaccessible to antibody.

Circulating antigen

Some tumour antigens have a circulating form in addition to the tumour-bound component. This has proved useful in the clinical situation for monitoring conventional chemotherapy, or for screening for primary or recurrent cancer (33). Tumour antigens which are commonly used for this purpose include carcinoembryonic antigen (CEA), α fetoprotein (AFP) and human chorionic gonadotrophin (HCG). Circulating antigen can however pose problems in antibody targeting as circulating antibody may bind the secreted antigen systemically, making it less available for tumour localisation (34;35)

Antigen location and accessibility

Antigens may be cell surface bound, or intracellular. Intracellular antigens will require internalisation of the antibody for effective targeting whereas this is not required for cell surface antigens. The distribution of the antigen within the tumour is also important as antigens expressed adjacent to tumour vasculature are also much more accessible from the systemic circulation than antigens in deeper parenchymal regions (36;37). In addition, antigens are often heterogeneously expressed within tumours (38-41) and this is one factor which makes antibody unlikely to target each cell within a tumour.

1.1.6 Tumour characteristics that influence antibody binding

There are significant physical barriers to the movement of whole antibodies through malignant tissue, particularly in solid tumours. Such factors reduce the

ability of antibodies to reach their target in vivo, limits their absolute uptake and also results in heterogeneous uptake (42). Since antibodies can only reach cancer cells by passing through the vascular and interstitial compartments three physiological factors restricting their movement have been identified (43). Heterogeneous blood supply as a result of vascular density and blood flow differences within different regions of a tumour limits the delivery of antibody to well-perfused regions of a tumour (44;45). Many tumours have elevated interstitial fluid pressure relating to the absence of any lymphatics which leads to an experimentally verifiable, radially outward convection in the tumour periphery which opposes inward diffusion of antibodies (46-48;48). Finally large transport distances in the interstitium increases the time required for slowly moving antibodies to reach distal regions of the tumour (49). As a result whole IgG escapes only slowly from the vasculature into tumour and in addition demonstrates slow penetration through tumour (50) tending to remain in peripheral areas of tumour close to blood vessels (51;52).

1.1.7 Immunotherapy of Cancer

One way in which antibodies have been used to treat cancer involves enhancing antibody directed host responses against tumour cells, so called immunotherapy. Immunotherapy was used as early as 1895 when Hericourt and Richet treated patients with anti-tumour serum resulting in some tumour responses (53). Early production of antibodies was made by immunising a suitable animal recipient, such as a mouse, with tumour antigen with subsequent removal of serum which contained antibody to the injected antigen. However the resulting polyclonal antibodies were produced by a heterogeneous population (i.e. different clones)

of B-lymphocytes and as a consequence had heterogeneous specificity and affinity which limited their suitability for clinical use. If individual B-lymphocytes from these antibody-producing cells could be isolated and cloned, each clone would produce a single species of antibody molecule that would bind to the same epitope. However in the past large scale production of these monoclonal antibodies was not possible because of the short survival of normal B-lymphocytes in culture. However this problem was solved by the discovery of the hybridoma technique by Kohler and Milstein in 1975, in which normal B-lymphocytes were immortalised by fusion with myeloma cells in culture (54). The resulting “hybridomas” can be screened subsequently using radioimmunoassay for single-cell clones capable of producing a specific antibody directed against a particular antigen. These selected clones can then be grown for monoclonal antibody mass production either in cell culture or in the peritoneal cavity of mice. Because these antibodies are produced by mouse B-lymphocytes, they are called murine monoclonal antibodies and since they bind to a single defined epitope with constant specificity and affinity are more suitable for therapeutic use. Since the 1980’s monoclonal antibody therapy, either alone or conjugated to therapeutic radionuclides, drugs, toxins or growth factors have been recognised as emerging agents for the diagnosis and treatment of cancer.

Early endeavours using mouse monoclonal antibodies met with only modest degrees of success, though their use was generally associated with minimal adverse effects primarily limited to mild allergic reactions. An initial trial conducted by Nadler et al using an antibody directed against a lymphoma

associated antigen lead to a reduction in circulating tumour cells without any associated significant toxicity (55). Subsequently Miller, Levy and colleagues developed patient specific anti-idiotypic antibodies directed against the unique immunoglobulin expressed on the surface of malignant B lymphocytes (56). The first example of an anti-idiotypic murine monoclonal antibody being used therapeutically in a patient with poorly differentiated lymphocytic lymphoma was reported in 1982 (56). After being treated with 400 mg of anti-idiotypic antibody the patient obtained a complete remission from their disease (56). This remission lasted for 6½ years and reproducible effects using such antibodies have been seen in other patients (57). However widespread clinical use was hampered by the prohibitive expense and complexity of developing patient specific antibodies. However this work provided proof of principle that targeting cells with antibodies could offer effective therapy and paved the way for modern monoclonal antibody directed therapies.

1.1.8 The problem of antibody Immunogenicity

Early in their development it became clear that a significant impediment to effective immunotherapy using murine monoclonal antibodies was the development of a host immune response directed against the therapeutic antibody. This usually occurs 2-3 weeks after the first antibody administration and within hours or days after repeated exposure. Although most of these immune responses are directed against the constant regions of the antibody, anti-idiotypic responses directed against the antigen binding sites may also occur. This antigenicity, leading to the formation of a human anti-mouse antibody (HAMA) immune response, has significant implications for the effectiveness of

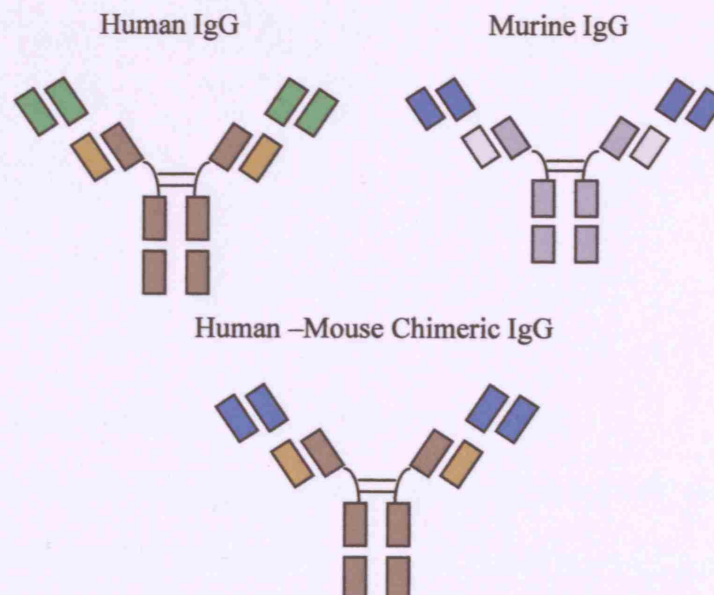
therapy. Firstly this antigenicity is thought to be responsible for the short serum half-lives of therapeutically administered murine antibodies. The clearance half-life of such antibodies is generally in the range of 18-48 hours, whereas physiologically produced antibodies may have circulating times of many weeks (19). Secondly, antibody use, particularly if repeated, may lead to immune allergic reactions including fever, rigors and chills during or immediately after the infusion of antibody and in more severe cases bronchospasm, hypotension, angioedema and acute lung injury (58). Although symptoms nearly always respond to supportive care (including stopping or temporarily slowing the infusion, intravenous fluids and the administration of intravenous hydrocortisone and/or an antihistamine), such responses mean that the presence of neutralising antibodies is a contraindication to further antibody therapy. A further disadvantage of such antibodies is that the murine constant regions have a reduced ability to harness human host effector mechanisms.

1.1.9 Reducing the immunogenicity of antibodies

Various approaches have been taken to reduce HAMA formation following therapeutic antibody administration. The use of immunosuppressants has been shown to be effective in delaying the emergence of neutralising antibodies, but it does not eradicate their formation following their cessation (59;60). With the advent of genetic engineering another approach that has been taken is to reduce the amount of foreign protein in antibodies by varying degrees of humanisation. An additional advantage of this approach is that it may also increase the ability of bound antibody to harness host effector mechanisms. Chimeric antibodies combining human and murine antibodies are produced by cloning the variable

region genes of a murine monoclonal antibody into a human expression vector containing the appropriate human constant region (61;62). Chimeric antibodies retain murine antigen binding sites but replace murine constant regions with human ones, increasing the proportion of human protein to 65-95%, and are illustrated in Figure 1.3. Such antibodies are less immunogenic than their murine counterparts yet retain the antigen binding affinity of the parent antibody (63). Chimeric antibodies can be given repeatedly usually with no significant reduction in circulating half-life. The half-life of chimeric antibodies in humans is much longer than murine antibodies, with half lives in serum of ten days or longer being reported (64;65). However significant HACA (human anti-chimeric antibody) formation may still be seen directed against the remaining murine regions (66).

Figure 1.3 Schematic representations of human-mouse chimeric antibodies



A further degree of humanisation is also possible by grafting the complementary determining region (CDR) of murine antibodies onto a structurally

complementary antibody scaffold of human origin (61). Although this humanisation process may be associated with reduced binding affinity, such antibodies have further reduced immunogenicity compared to chimeric antibodies (67). Fully humanised antibodies are a further development and are obtained from either screening of phage libraries or the use of transgenic mice containing human immunoglobulin genes. However even in these human antibodies, the specificity-determining residues (SDR's), which are the residues of the CDR that are most critical in antigen-antibody interactions, remain potential targets for the immune system. As a result there may still be rapid clearance of antibody through the anti-idiotypic response, a part of the natural feedback mechanisms of the immune system (68).

Table 1.1: The Increasing Humanisation of Whole Monoclonal Antibodies. This aims to reduce their immunogenicity and also increase their ability to harness host effector mechanisms. Antibodies produced against different antibody types are referred to as being either human anti-mouse (HAMA), human anti-chimeric (HACA) or human anti-human (HAHA).

Antibody Type	Production	Advantages	Limitations
Murine	Hybridoma technique	High affinity, ease of production	Poor at eliciting host responses, HAMA formation
Chimeric	Recombinant DNA technology	Lower immunogenicity cf. murine counterparts	HACA still occurs to murine components
Humanised	CDR grafting onto human IgG backbone	Further reduced immunogenicity cf. chimeric antibodies	Reduced specificity and affinity due to additional manipulation
Human	Phage display library screening or transgenic mice	Eliminated immunogenicity	De-novo generation necessary cf. modification of well known murine counterparts

1.1.10 Clinical Immunotherapy of Malignant Disease

With the development of humanised and human antibodies with reduced immunogenicity, augmented effector functions and improved pharmacokinetics combined, along with the selection of more effective antigenic targets, modern immunotherapy has achieved considerable success, particularly in the treatment of the lymphomas.

Table 1.2: Examples of naked monoclonal antibodies currently licensed for clinical use or under development. Modified from (69)

Target antigen	Cancer type	Antibody	Antibody type
CD20	Non Hodgkin lymphoma	Rituximab	Chimeric
CD22	Non Hodgkin lymphoma	Epratuzumab	Humanised
HLA-DR	Non Hodgkin lymphoma	Remitogen	Humanised
HER-2/neu (ERBB2)	Breast	Trastuzumab	Humanised
CD33	Acute myeloid leukaemia	Gemtuzumab ozogamicin	Humanised
CD52	B-cell chronic lymphocytic leukaemia	Alemtuzumab	Humanised
Epidermal growth factor receptor	Colorectal, head and neck, non-small cell lung cancer	Cituximab	Chimeric
Vascular endothelial growth factor	Solid tumours	Bevacizumab	Humanised
VEGFR2	Solid tumours	IMC-1C11	Chimeric
A33	Colorectal cancer	huA33	Humanised
G250/MN	Renal cell carcinoma	G250	Chimeric

1.1.11 Antibodies as a carrier of cytotoxic agents.

Naked antibody use has been associated with impressive single agent activity in the treatment of lymphoma, a tumour type known to have an increased sensitivity to immunomodulation. However for other disease types, antibodies have generally shown only modest activity in isolation, with significant activity usually only seen when they are given in combination with additional systemically administered cytotoxic agents. In addition, theoretical barriers to the efficacy of naked antibody approaches include reliance upon host immune effector mechanisms, incomplete tumour targeting resulting from antigen negative tumour cells, heterogeneous antibody distribution within tumours, and

the redundancy of tumour growth signalling pathways. Another approach therefore that has been adopted to increase response rates is the conjugation of antibodies with cytotoxic agents including chemotherapy, plant toxins and radionuclides.

1.1.12 Targeted Chemotherapy

Antibodies may be used to target chemotherapeutics specifically to tumours. Many anti-cancer drugs including Adriamycin (70), Methotrexate (71), Melphalan (72) and Daunorubicin (73) have been successfully conjugated to monoclonal antibodies for selective tumour delivery. However, to date clinical success has been limited due to the low levels of active drug that can be delivered to sites of tumour, or a loss in drug potency and antibody binding following conjugation (74).

However high potency cytotoxics conjugated to antibodies have been more promising. One such cytotoxic that has been used is Calicheamicin, a potent anti-tumour antibiotic that cleaves double stranded DNA (69). Gemtuzumab ozogamicin is a humanised-antibody chemotherapeutic agent that targets the CD33 antigen. CD33 is present on myeloid blasts in 80% of patients with acute myeloid leukaemia and is expressed upon maturing haematopoietic-progenitor cells, but is not present on healthy stem cells. Gemtuzumab ozogamicin consists of calicheamicin joined by a linker to anti-CD33 monoclonal antibody. On binding to CD33, the construct is rapidly internalised into the cell and active drug is released when the construct reaches lysosomal pH. The drug has shown activity in patients with non-secondary acute myeloid leukaemia and was licensed by the U.S. Federal Drug Authority in 2000. However, although theoretically toxicity should be limited to the targeted cell population, some

systemic toxicity has been observed in its use including grade 3 and 4 neutropenia and thrombocytopenia along with transient and reversible hepatic toxicity. These were less than might be expected with the drug used systemically (75).

Antibody directed enzyme prodrug therapy (ADEPT) is an alternative pretargeting strategy to allow increased amounts of chemotherapeutic agent to be produced at sites of tumour (76). In this system an enzyme is conjugated to an anti-tumour antibody and subsequently, following clearance of the conjugate from the circulation, a non-toxic prodrug is administered. This is then converted to the active cytotoxic drug by the enzyme located at the tumour site. Since one enzyme molecule can activate many drug molecules ADEPT may overcome the problems of inadequate amounts of cytotoxicity at sites of tumour.

1.1.13 Targeted Toxins

Another approach that has been taken is the use of antibodies to deliver plant or bacterial toxins, highly active molecules that enzymatically inactivate protein synthesis leading to cell death. Although immunotoxins generally need to be internalised into the cell for effect as they act upon intracellular targets such as ribosomes, only a few molecules are needed for effect due to their very high potency (77). The majority of clinical trials have incorporated the Ricin A chain as the toxin but their success has been limited by a potentially lethal vascular leak syndrome (78-80). More recently a phase I trial has been reported in which a toxin linked IL-2R α targeting antibody has been used in the treatment of chemoresistant haematological malignancies (81). LMB-2 is a recombinant

immunotoxin which contains the variable light and heavy domains of an anti - CD25 monoclonal antibody fused by a peptide linker to PE38, a truncated form of *Pseudomonas* exotoxin which has had its native binding domain removed. In this study of 35 patients with various haematological malignancies a single patient with hairy cell leukaemia attained a complete remission which was sustained at 20 months and seven other patients achieved partial responses. All four patients with hairy cell leukaemia responded to the immunotoxin therapy (82). Toxicity was mild and consisted of transient transaminitis and fever and unlike many previous studies utilising immunotoxins vascular leak syndrome was not seen. One theoretical problem with immunotoxins however is that there is no crossfire effect, so theoretically each tumour cell would need to be targeted in order to produce 'cures'.

1.1.14 Targeted Radiotherapy

Another approach using conjugated antibodies has been to carry therapeutic radionuclides, so called radioimmunotherapy (RIT). Radiation is perhaps the most studied cytotoxic agent used for the treatment of malignant disease and external beam radiotherapy (EBRT) has proved remarkably effective in sterilising localised micrometastatic and small volume localised disease in both radiosensitive tumours such as the lymphomas and also the more radioresistant common epithelial tumours.

1.2 Radioimmunotherapy

1.2.1 General considerations

A number of factors need to be considered when using antibodies to deliver radiation to tumour. An important concept in RIT is its ability to cause an effective crossfire effect, i.e. the irradiation of untargeted cells by radionuclide bound to neighbouring targeted cells due to emitted radiation. As a result radionuclides that can provide effective crossfire effect offer considerable advantages in terms of tumour kill by overcoming the problem of heterogeneity of antibody delivery. The amount of crossfire that will occur during RIT is dependent upon the radionuclide chosen, of which there are a large number that emit a range of radioactive particles with ranges from nanometres to millimetres. In addition 'bystander' effects following irradiation may also result from the effects of irradiated cells upon unirradiated cells (83;84).

However these crossfire effects, which are so beneficial in terms of tumour cure, also lead to the irradiation of surrounding normal tissues. The doses of radiation that can be delivered safely to tumours by RIT are often therefore considerably less than those possible in either EBRT or brachytherapy due to dose limiting marrow toxicity, irradiated whilst antibody remains in the circulation. As a result, in non-myeloablative RIT tumour absorbed doses are often much less than those commonly used in EBRT.

Furthermore, during RIT, radiation is delivered at much lower dose rates than either EBRT or even the 50 cGy per hour characteristic of 'low dose rate' brachytherapy. In RIT dose rates are commonly less than 10-20 cGy per hour and in addition exponentially decrease with time. With the commonly used β

emitting radionuclides, dose rate is a significant modifier of the biological effects observed in tissues due to a dose rate effect.

Finally many years of external beam radiotherapy indicate that a clear relationship exists between radiation absorbed dose and early and late normal tissue toxicity and tumour response, even for specific tumour types (85;86). In RIT absorbed doses are determined primarily by antibody pharmacokinetics rather than radiation field arrangements as in EBRT. This means that in RIT estimating absorbed dose is a complex process due to heterogeneity of radionuclide distribution and the complex time patterns of disappearance of activity. However an ability to predict absorbed dose distributions in normal tissue and tumours is necessary to allow safe and optimally delivered RIT.

1.2.2 Radionuclide Choice

The killing of tumour cells during RIT is most effectively achieved by emitted particulate radiations which deposit the majority of their ionising energy within the targeted tumour. In RIT another important consideration is the pharmacokinetic properties of the antibody in relation to the half-life of the attached radionuclide. For example, following the administration of whole antibodies, effective tumour localisation may take several days and there would be little point in combining such antibodies with radionuclides with a decay half-life of only a few hours. In terms of radionuclide half-life, it has been suggested therefore that for optimal effect this should match the biological half-life of the antibody in tumour (87).

A large number of radionuclides exist and the physical characteristics of a selection of radionuclides that have been used in RIT are shown in Table 1.3.

Table 1.3: Physical characteristics of potential radionuclides for RIT

Radionuclide	Half-life	Radiation type	Energy MeV	Maximum particle range
^{131}I	8 days	β	0.6	2.0 mm
		γ	0.364	
^{90}Y	2.7 days	β	2.28	12 mm
^{67}Cu	2.5 days	β	0.54	1.8 mm
		γ	0.185	
^{177}Lu	6.7 days	β	0.497	1.5 mm
		γ	0.208	
^{153}Sm	1.95 days	β	0.8	3.0 mm
		γ	0.103	
^{186}Re	3.77 days	β	1.08	5.0 mm
		γ	0.131	
^{188}Re	16.95 hours	β	2.13	11 mm
		γ	0.155	
^{211}At	7.2 hours	α	6.8	65 μm
^{212}Bi	1.0 hours	α	7.8	70 μm
		γ	0.72	
^{125}I	60 days	Auger	0.027	10 nm

It can be appreciated therefore that the amount of crossfire that will occur during RIT is dependent upon the radionuclide chosen. The particle range of long range β emitters such as ^{131}I and ^{90}Y means that considerable cross-fire will occur with possible sterilisation of untargeted cells and has been one reason for their extensive use in RIT. Radionuclides emitting α particles have a much shorter range in tissue compared to β emitters which leads to a reduced cross-fire effect, though this is not completely absent. As a result in order to deliver a

homogenous dose to tumour, antibody distribution must be similarly homogeneous. Auger emitters are radionuclides with even shorter range emissions. They are produced when a nuclide decays by electron capture and triggers quantum effects including emission of orbital electrons (Auger electrons) and soft γ rays. Characteristically, Auger electrons are ultra-short range (approximately 1 μm) and are of high LET quality. Cell sterilisation results only if Auger-emitters can be conveyed to the cell nucleus (88) and with antibodies this only occurs if they are internalised by the cell (89).

With regards to tumour irradiation the optimal radionuclide will also depend upon tumour size with relatively increased losses of radiation from a tumour if the radiation path length is too long. Using β emitting radionuclides it has been estimated that the optimal tumour size for curability is 3 mm for ^{131}I and 3 cm for ^{90}Y (90). The short path lengths of α and Auger emitters, although a potential disadvantage for the treatment of solid tumours, may theoretically be advantageous in the treatment of micrometastatic disease by the same reasoning. Since in clinical practice patients may have many tumours of differing sizes, it has been proposed that further optimisation of RIT might come from the use of antibodies labelled with 'cocktails' of radionuclides emitting radiation of differing path lengths (87).

Many radionuclides also emit highly penetrating gamma radiation which contributes little to the tumour absorbed dose, but contributes significantly to the whole body radiation burden borne by patients. For example dosimetric calculations for clinical RIT using a ^{131}I labelled antibody suggest penetrating γ

radiation contributed 50% of the dose received by the bone marrow with the other 50% coming from the non-penetrating radiation (91). Similar calculations based on animal data and extrapolated to man resulted in a value of 30% as the contribution of penetrating radiation to the bone marrow dose (92). The other commonly used radionuclide ^{90}Y benefits from an absence of gamma radiation, which reduces whole body radiation, but also has a higher energy β particle which along with a predilection for accumulation in bone leads to higher bone marrow irradiation (93). Alternatives to ^{131}I and ^{90}Y include ^{186}Re or ^{67}Cu , both of which would reduce the whole body absorbed dose due to their reduced gamma emissions. In a study looking at patients with NHL treated with either ^{67}Cu , ^{90}Y or ^{131}I labelled Lym-1 antibody, although the mean dose to tumour from the ^{131}I and ^{90}Y labelled antibody was higher, the radiation dose to the whole body and marrow was also higher leading to a higher therapeutic index for the ^{67}Cu labelled antibody (94).

Regardless of the type of radiation emitted by the various radioisotopes used for RIT they can be grossly divided into two categories on the basis of their chemical characteristics: halogens (e.g. ^{131}I or ^{211}At) and radiometals (^{90}Y , ^{186}Re). In general, halogens, ^{131}I in particular, can be labelled easily and directly using either the iodogen or chloramine T methods (95;96), whereas radiometals require chelators such as benzyl-diethylenetriaminepentaacetic acid or 4,7,10-tetra-azacyclodecane-N, N', N'', N''' tetraacetic acid (DOTA) which are conjugated to the antibodies to allow suitable antibody labelling (97;98).

In this thesis ^{131}I has been used to label both antibodies under investigation.

Reasons for using ^{131}I in these studies are multiple and include ready availability, a useful γ emission allowing for detailed biodistribution studies to be performed and simple radiolabelling in comparison to radiometals.

1.2.3 Radiation delivery by RIT

Radiation delivery to normal tissues and tumour following RIT differs in a number of ways from that achieved by fractionated EBRT. In the first instance radiation effects are systemic in that although treatment is targeted most tissues in the body are exposed whilst antibody is in the circulation. Secondly radiation is often delivered at a much lower dose rate than that used in fractionated EBRT or even low dose rate brachytherapy. It is well known that radiation delivered at high dose rate is more biologically effective than that delivered at low dose rate. Furthermore, due to heterogeneous antibody distribution within tissues, radiation delivery is often more spatially variable than for either EBRT or brachytherapy. In order to understand the effects of these differences a basic understanding of radiobiology is required.

1.2.4 General Principles of Radiobiology

Radiation works by producing breaks in the DNA of cells. Single strand breaks are of little consequence because cells have efficient mechanisms for repairing them, whereas double DNA strand breaks are generally non-repairable (99). Without intact templates for their mutual repair, double strand breaks may be misrepaired and disrupt the integrity of the chromosome. Cell death usually ensues at the first or subsequent mitotic divisions as a result of proliferative cell death or secondary apoptosis resulting directly from the unrepaired DNA strand

breaks (100). Thus in normal tissues and most tumours, radiation responses occur at a rate proportional to their rate of proliferative turnover. For example the rapidly proliferative cells of the mucosa of the respiratory and gastrointestinal tracts and the red marrow all develop a detectable reaction within days or weeks of radiation exposure; such effects are termed early radiation effects. By comparison late radiation effects are found in tissues with a slow cell turnover, such as connective tissue, kidney, cartilage and bone and may be delayed by many weeks or months. The rate of appearance of injury also depends upon the proliferative activity of 'stem' or germinal cells and the lifetime of the differentiated progeny of these cells.

The amount of radiation damage in tissues is dependent upon the absorbed dose, but also the type of radiation to which they are exposed. Linear energy transfer (LET) is the average energy locally imparted to tissue by a charged particle of specified energy per unit distance traversed. Low LET radiation is typified by β particles and γ radiation, whereas α and Auger particles possess a high LET. Each Gray of low LET radiation produces about 1000 initial single strand breaks and about 40 double strand breaks in nuclear DNA whereas high LET radiation is more damaging.

1.2.5 Dose Rate Effects and the Linear Quadratic Model

Most therapies in oncology, including RIT, utilise low LET radiation, either as γ or X rays during EBRT or β particles such as those emitted from ^{131}I during the therapy of thyroid malignancies. Early in its use it was noted that if radiation was given more slowly over a longer time course, or as a series of fractionated

doses, an amplification of the therapeutic difference between normal tissues occurred. In the 1920's Regaud et al in a pioneering set of experiments using the testis of the rat as a model tumour system, showed that only through the use of multiple radiation exposures could animals be completely sterilised without producing severe injury to the scrotum (101-104). This 'fractionation' of radiotherapy was soon tested in the clinic by Coutard, who first used fractionated radiotherapy for the treatment of head and neck cancers with spectacularly improved results (105;106). Largely as a result of these and related experiments, fractionated treatment subsequently became the standard form of radiation therapy and the biological basis for this is currently best understood in terms of the linear-quadratic formulation. This model proposes that cell survival results from cell killing in the nucleus as a result of either single or double hit events in the nuclear DNA (107;108). In the first type of event a single ionisation is sufficiently potent to create a lethal double stranded DNA lesion. In the second type of event, lethal damage results from the interaction of two single strand (sub-lethal) DNA lesions, each of which is induced by a separate ionising interaction. Since sub-lethal damage may be repaired with time, the amount of lethal type B damage is dependent upon the temporal frequency of ionising events in the vicinity of any critical DNA target and gives rise to a 'dose rate effect'. At lower dose rates there is a greater opportunity for an initial sub-lethal event to recover before being added to by a second event, hence reducing the incidence of lethal damage.

1.2.6 The Importance of α/β Ratios

The response of individual tissues and tumours to low LET radiation is highly variable and can be described in terms of α/β ratios. Douglas and Fowler developed a novel method of data analysis called the reciprocal dose plot which allowed α/β to be derived for different tissues (109). An understanding of α/β ratios is fundamental when applying the linear quadratic model. In essence they are the parameters which, for a specified tissue or effect, defines the relationship between α , which represents intrinsic radiosensitivity and β , potential sparing capacity. Typical α/β ratios for a selection of normal tissues and tumours is shown in Tables 1.4 and 1.5 and the effect of radiation dose rate on tissues with differing α/β ratios is shown in Table 1.6.

Table 1.4: Representative α/β ratios for normal tissues (99)

Tissue type	Endpoint	Time of Response	α/β ratio
Skin	Erythema	Early	10.6
Lung	Pneumonitis	Early	8.8
Oral mucosa	Mucositis	Early	10.8
Red Marrow	Aplasia	Early	10
Skin	Telangiectasia	Late	2.7
Skin	Fibrosis	Late	1.7
Lung	Fibrosis	Late	< 3.8
Bowel	Perforation/ stricture	Late	3.9
Spinal cord	Myelopathy	Late	< 3.3

Table 1.5: Representative α/β ratios for different tumour types (110)

Tumour Type	α/β ratio
Melanoma	4.8
Squamous Carcinoma	6.1
Adenocarcinoma	5.7
Lymphoma	8.8
Small Cell Lung Carcinoma	8.0

Table 1.6: Summary of the linear quadratic model parameters and concepts

Tissue Type	α/β ratio	Dose response Curve shape	Dose Rate Sensitivity
Early responding normal tissues and most tumours	High	Steep initial slope (α is high)	low
Late responding normal tissues	Low	Shallow initial slope (α is low)	High

1.2.7 Specific Radiobiology of RIT

All types of radical radiotherapy ultimately aim to maximise damage to the tumour for a minimal amount of normal tissue damage i.e. to achieve the highest possible therapeutic ratio. It can be seen therefore that the highly fractionated, low dose rate radiation typical of RIT will spare late responding normal tissues such as connective tissue and spinal cord with toxic effects being seen in those with a high α/β ratio, such as bone marrow. In addition tumours with a low α/β ratio, such as the common solid tumours, will tend to be spared by highly fractionated radiation more than those with a high α/β ratio, such as lymphomas and small cell lung cancer (111). It has been estimated however that given the already highly fractionated nature of traditional EBRT, RIT is probably not more than 20% less effective than conventional radiotherapy (112).

However at the very low dose rates delivered by RIT, cell division can often continue in tumour leading to repopulation *during* therapy. As a result in RIT in which low dose rates of irradiation are exponentially decreasing, tumour cell sterilisation takes place in a number of distinct phases. In the first phase at the highest dose rates, cell cycling will be inhibited and tumour cells will be sterilised in direct proportion to absorbed dose (assuming exponential survival

curves). As the dose rate decreases however, limited cell cycling could take place, perhaps with a block occurring in G₂ which may be advantageous to cell killing by an 'inverse dose rate effect'. Finally as the dose rate decreases further, progression is enabled and tumour repopulation becomes possible (113). An enormous range of dose rates have been reported in the literature to 'just stop' proliferation in a variety of cells with the highest rates required for fast proliferating cells in culture, and also in cells with the highest radioresistance (114).

1.2.8 The Inverse dose rate effect

Radiation-induced cell damage activates both G₁ and G₂-phase checkpoints in the cell cycle which leads to prolonged G₁ and G₂ phase arrest. During normal physiology these safety mechanisms allows more time for DNA damage to be repaired before cell replication (115;116). However since cells in G₂/M are more sensitive to radiation than cells in other phases of the cell cycle, it is also thought that increasing the number of cells in this more radiosensitive phase could increase the relative effectiveness of radiation by the order of 20-30% (114). This 'inverse dose rate effect' is thought to be an important mechanism for increasing the efficacy of RIT, particularly in the treatment of lymphomas which have a propensity to undergo programmed cell death or apoptosis. In several different experiments, a variety of lymphoma cell lines exposed to continuous LDR radiation, as well as to exponentially decreasing radiation typical of RIT, have been observed to arrest in the G₂M phase of the cell cycle and then undergo apoptosis (117-120). In one study caffeine, which affects cell cycle distribution by reducing G₂M phase arrest was found to reduce LDR

radiation induced apoptosis, whereas prolonging G₂M phase arrest by Nocodazole enhanced apoptosis (119). However whether such an effect is truly observed in clinical practice is difficult to know given the heterogeneous cell population observed in tumours where many cells are non-cycling.

1.2.9 Low dose hyper-radiosensitivity

In RIT radiation is characteristically delivered at much lower dose rates than that characteristic of EBRT and the doses delivered to tumour are also often much lower. Low dose hyper-radiosensitivity (HRS) is the term used to describe the experimental observation that, following single radiation doses of less than 0.5 Gy, more effective killing of clonogenic cells per unit dose is observed than at higher doses, whilst increasing dose above 0.5-1 Gy leads to increased radioresistance (IRR). Following the first demonstration of HRS and IRR in mammalian cells by Marples and Joiner in 1993 (121) the phenomenon has now been observed in a large number of cell lines and normal tissues and verified in a number of different laboratories (122). HRS appears to be the default survival response of cells to radiation injury at doses below 10-20 cGy (122) with increasing levels of radiation-induced damage activating a protective response resulting in IRR. It has been speculated that it is beneficial for an organism to allow small numbers of cells with low levels of damage to die rather than risk mutation through inaccurate repair and survival (123). The exact molecular mechanisms underlying HRS/IRR are not conclusively identified and are the subject of ongoing research, but it has been suggested that continuous, very protracted courses of low dose rate irradiation, at rates less than 10 cGy per hour, might result in enhanced cell killing (122). Given that RIT is known to

lead to G2 cell cycle arrest it is interesting that HRS/IRR is known to be a phenomenon predominantly seen in G2 phase cells (124;125) and therefore might be a significant factor in increasing the efficacy of RIT.

1.2.10 Re-oxygenation and hypoxic radioresistance

Hypoxia is a feature of many solid tumours and since oxygen is known to enhance cell killing by radiation it is an important determinant of curability (126). The exact mechanism of this oxygen effect is unknown, but the 'radical competition model' suggests that oxygen acts as a radiosensitizer. It does so by forming peroxides in important biomolecules already damaged by radiation exposure thereby 'fixing' the radiation damage. In the absence of oxygen, DNA can be restored to its pre-irradiated condition by hydrogen donation from endogenous reducing species in the cell, such as glutathione (99). As a result even a small proportion of hypoxic cells will limit the radiocurability of a tumour. It is generally believed that a process of re-oxygenation of hypoxic cells between treatment fractions is one of the factors that leads to the success of conventional EBRT (99;127). During the interval between dose fractions, killed normoxic cells are eliminated and previously hypoxic cells gain better access to oxygen. However in RIT where most of the radiation dose is delivered within a few days and is continuous, it is possible that incomplete re-oxygenation may occur leading to an increased likelihood of treatment failure (128).

1.2.11 Dose heterogeneity

In RIT, antibody biodistributions in tumour are usually heterogeneous and often leads to a heterogeneous distribution of absorbed doses and dose rates within

different parts of tumour. In one study looking at Raji B-cell lymphoma xenografts using intratumoural micro-thermoluminescent dosimeters, the measured absorbed dose in tumour varied by up to 400% using an ^{131}I radiolabelled antibody (129). This data is supported by theoretical dose calculations made using autoradiography (20;130-132). As a result dose heterogeneity is common within tumours even when using a radioisotope which benefits from significant cross-fire effects. Although selective targeting of viable cells in well perfused areas of the tumour will allow for effective cell killing in these areas (29), tumour re-growth will be determined not by these cells but by the clusters of tumour cells which are less well targeted and that receive a reduced total dose at lower dose rates.

1.2.12 Normal bone marrow function and Haematological toxicity following RIT

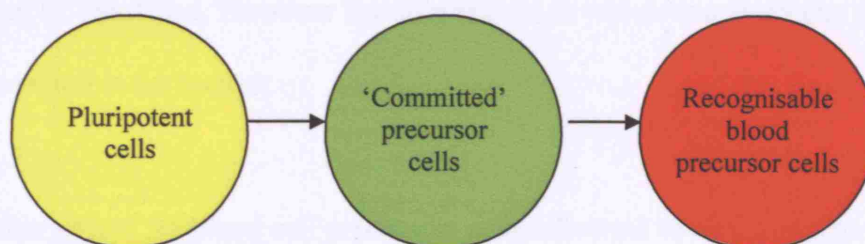
The bone marrow is dose limiting in RIT and toxicity is manifest as both acute and chronic blood cytopenias. The response of the bone marrow to irradiation is complex and depends upon the response of mature blood constituents but also marrow stem and stromal cells. Red marrow output may be increased dramatically in response to blood loss or infection and equally reduced in cases of excess, such as experimentally induced polycythaemia. These changes may be achieved with no change in the numbers of haematopoietic progenitor cells in the marrow. In addition normal blood cell production may be maintained even when the number of progenitor cells has been severely reduced as a result of cytotoxic therapy (133). As a result, the numbers of peripheral blood cells gives little indication of changes in the number or quality of the progenitor cells in the

marrow which survive cytotoxic insult. Equally the recognisable blood cell precursors in the marrow do not provide any guide to the functional state of their progenitor cell population.

1.2.13 Red marrow cell populations

The production of blood cells can conveniently be divided into 3 stages (134) as shown in Figure 1.4. Pluripotent stem cells have the ability to differentiate into any of the specialised blood cells whereas 'committed' precursor cell populations have a determined and unchangeable development path eventually leading to mature blood elements. Cells entering the second two stages of the production line are capable of undergoing several divisions during the time in which they are maturing into the final end product of each stage and the number of divisions appears to be flexible so that the number of cells formed may be increased, for example under conditions of high cell demand.

Figure 1.4: Bone marrow cellular hierarchy



The pluripotent stem cells are ultimately responsible for maintaining production of blood cells. Unfortunately in all mammals except mice there is no technique by which these cells can be identified and it is therefore necessary to use the earliest precursors of the functional blood cells as an indicator of their function.

In vivo and in-vitro assays of early cell precursors all depend upon the development, under specific conditions, of a clone of cells. In addition marrow function is also dependent upon a healthy marrow micro environmental milieu both for the survival of the stem cells and for regulation of their differentiation, maturation and proliferation. In a similar way the function of these marrow stromal cells may be measured using in vitro assays.

1.2.14 Assessing red marrow function: in vitro colony assays.

The Spleen colony-forming unit assay (CFU-S) was first described by Till and McCulloch in 1961 and assays a true pluripotent stem cell (135). The assay is performed by injecting intravenously a small number of marrow cells into a mouse which has been irradiated to a dose leading to myeloablation. 8-12 days later the mouse is killed and its spleen removed and fixed. Within the spleen, visible nodules can be seen which represent colonies of cells produced from pluripotent stem cells in the injected marrow and counting these gives a quantitative measure of their number. Using this technique it is possible to measure survival and plot recovery of the most primitive haematopoietic cell that can be identified. However the only animal in which this assay can reliably be measured is the mouse.

All other assays for blood cell precursors are performed *in vitro* and depend upon the development of colonies by appropriate stimulation of the precursor cells wished to be identified using the appropriate growth factors. In general, a cell suspension containing a defined number of cells is added to a methylcellulose or agar solution which subsequently sets to form a semi-solid

support medium. This is then incubated for a defined period and the resulting cell colonies counted to give an indication of viable stem cell numbers. Viable colonies are generally defined as a group of at least 50-100 cells.

Morphologically distinct colonies may be seen reflecting erythroid (BFU-E from the erythroid 'burst' forming cell) and granulocyte or macrophage differentiated cells (CFU-GM). Although these assays do not measure the same entity as the CFU-S assay, measuring changes in these very early committed progenitor cells has been shown to reflect changes in the numbers of CFU-S as judged from comparisons with CFU-S measurements made in mice. A significant advantage of these techniques is their relative simplicity and applicability to all mammals including man (136). Similar types of assays are used to assess the haematopoietic microenvironment using a fibroblast colony-forming unit assay (CFU-F) (137).

1.2.15 Marrow response to ionising radiation

Following irradiation, changes in cell count are dictated by the response of marrow stem cells but also the survival of the mature blood elements. As a result, although blood platelet and white cell counts may decrease rapidly following marrow irradiation, anaemia is less common. This is because the slow turnover of mature erythrocytes allows time for surviving haematopoietic precursor cells to compensate for injury before effects from radiation become apparent (138).

Marrow stem cells are acute responding tissues with a high α/β ratio. Dose response curves for CFU-S were first produced by Till and McCulloch (135).

These have now been measured by many investigators using ionising radiation of different types and a wide range of dose rates and demonstrate a high degree of consistency (139). Cell survival curves demonstrate a small shoulder indicating that cell killing shows little dependence upon variations in dose rate. This suggests that the cells have a limited ability to repair sublethal damage (140). Following irradiation there is rapid initial depletion of CFU-S followed by a lag in re-growth lasting 1-2 days. Subsequently there is a burst of regeneration with a mean doubling time of 28 hours. This is followed by slower recovery with a return to normal levels seen between 15 and 46 days for doses of 4.5 Gy or less. Final levels of CFU-S following such doses have varied between 70 and 100% of baseline. Irradiation at higher dose levels is associated with markedly prolonged recovery times, typically in the order of months, with further reduced final CFU-S levels.

The effects of protracted continuous low dose irradiation upon the survival of CFU-S versus accumulated dose has also been investigated by Wu and Lajtha (140). In this study they demonstrated that CFU-S survival was highly dependent upon dose rate and that at the lowest dose rates (<0.45 Gy/day) mice were able to survive for several weeks whilst absorbing doses in excess of 30 Gy whole body irradiation. This resulted from a large increase in the rate of proliferation of CFU-S and of an increased expansion of the 'committed' cell populations during their maturation.

Survival curves for CFU-GM demonstrate similar features to those outlined already for CFU-S (141). It can be appreciated therefore that progenitor cell and

marrow function will be spared little by the low dose irradiation typical of RIT. However the marrow has highly effective methods of repopulation and in the absence of marrow ablation, can recover from significant amounts of radiation, particularly if given at very low dose rates.

1.2.16 Clinical Radiation Dosimetry for RIT.

Radiation oncology studies conducted over several decades have shown that treatment planning for an individual patient can be used to optimise response and morbidity. Patient-specific calculations of radiation doses delivered to tumours and normal tissues are routine in external beam radiotherapy with calibration of linear accelerators using phantom and in-vivo dosimetry. This helps to assure the accuracy of external beam dose delivery. The intended radiation doses are determined by the response-morbidity relationships observed in studies of earlier patients, so that treatment planning actually represents careful definition of radiation dose distribution for each patient. Equally importantly, a vast body of human data indicates a clear relation between radiation dose and early and late normal tissue toxicity, and between radiation dose and tumour response even for specific tumour types (85;86).

Significant differences exist between radiation delivered as EBRT or RIT, necessitating different approaches to radiation dosimetry. However knowing the doses administered to normal tissues and tumour remains equally important to understanding their dose-response relationships. Early RIT trials were mainly pragmatic, without detailed treatment planning or pre-treatment estimates of anticipated radiation doses to either tumour or normal tissues. In most cases

radioimmunoimaging was primarily utilised in order to demonstrate adequate uptake of radionuclide in tumour deposits. However a more productive use of such biodistribution data is to obtain tumour and normal tissue radiation dosimetry. Hopefully such information will allow a prediction of normal and tumour toxicity, maximising the likelihood of safe and optimally effective treatment for each patient. Radiation dosimetry has now assumed a much greater importance and is required in both phase I and phase II studies, permitting either a retrospective assessment of normal tissue toxicities and tumour responses in relation to absorbed dose, or as a pre-treatment technique to allow individualisation of administered activity. This depends upon individual pharmacokinetics determined using tracer studies.

1.2.17 Quantification of activity in visceral organs and tumour during RIT

The development and validation of RIT by antibodies requires that the distribution of activity in the tumour and normal tissues is accurately estimated. In clinical studies however the measurement of activity in living tissues is rarely measured directly, whether radiation is given as EBRT, brachytherapy or RIT due to the invasive nature of even the smallest dosimeters. Therefore activity measurements have to be calculated rather than directly measured. In RIT, γ emitting radionuclides such as ^{131}I allow an estimate of the levels of radioactivity in tissues to be obtained non-invasively using a nuclear medicine gamma camera. Gamma cameras employ a large sheet of scintillation material coupled to a group of photomultiplier tubes, with a collimator between the patient and the scintillator. Collimators are typically made of lead and are about 4-5 cm thick, contain thousands of holes through which gamma rays are allowed

to pass, with photons coming from angles other than parallel to the camera being attenuated. A projection image of the object under study (organs within a patient) is developed after many thousands or millions of events strike the crystal and are processed. Drawing regions of interest (ROI's) allows counts to be taken from objects of interest, i.e. normal organs or tumour (142). Planar imaging can be used to estimate activity in various tissues and has been applied in RIT (143). In this method usually two images are taken from the front and back of the patient and the geometric mean of the counts is taken. Using such methods it is possible at specified times to estimate activity in identified regions of the body. External planar imaging with a gamma camera may be performed in less than 30 minutes. It requires no special hardware beyond that found in a standard nuclear medicine department and as a consequence has become the most widely used method for quantifying tissue activity (143).

However planar methods have difficulty in making accurate estimates of activity where there is significant activity in under or overlying tissues. Single photon emission tomography (SPECT) on the other hand has the potential to overcome this problem by providing three dimensional data. If the gamma camera heads are rotated around the body, with projected images taken at multiple angles around the body, 3D information may be reconstructed from these multiple images, using techniques common to topographic imaging in other modalities such as CT. This three dimensional information may be displayed as either slices or 3D renderings. This yields a more detailed understanding of the activity distribution and when combined with scatter and attenuation correction has been shown to be superior to planar imaging methods (144).

1.2.18 Quantification of activity in bone Marrow during RIT

Bone marrow irradiation leading to marrow toxicity is dose limiting in RIT and therefore the methods utilised for estimating marrow absorbed dose are particularly important. Such methods have traditionally addressed contributions from radionuclide sources in blood, body or both, but as a result of studies sponsored by the Dosimetry Task Group of the American Association of Physicists in Medicine, a standardised method for estimating marrow radiation dose has been produced. In this approach, the radioactivity in red marrow is assumed equal to the activity in blood, which can be simply measured, multiplied by a factor between 0.32 and 0.36 (145). This approach is considered adequate in the absence of specific marrow localisation of antibody, but where this does occur, a number of other techniques may be used. These include marrow imaging techniques (with ROI definition of marrow rich regions) (146;147) or direct marrow sampling. However sampling errors are a significant problem with both imaging and direct sampling techniques. In addition with direct marrow sampling considerable pain and discomfort occurs for the patient that limits the number of samples that can be obtained.

1.2.18 Radiation Dosimetry and Cumulated activity

Cumulated activity and the volume or mass of tissue in which it is distributed are the principal quantities required for radiation dosimetric calculations (148). The cumulated activity for radionuclides in tumours and other tissues are estimated by linking the serial quantitative activities and estimating the area under the curve. This can be performed in a number of ways including direct integration, with the most direct method being the trapezoidal method. However,

although simple to perform, this method presents problems when estimating activity beyond the last observed value. Another approach is least squares analysis in which an attempt is made to fit curves of a given shape to the data. The curves are represented by mathematical expressions which can be directly integrated, and with decaying radioactive sources the most common approach is to attempt to characterise the data using a series of exponential terms.

1.2.20 Population based dosimetry: The MIRD schema

Radiation dosimetry is the conversion of radioactivity into an absorbed dose and a number of methods are available to do this. The Medical Internal Radiation Dose (MIRD) committee has developed a system whereby an 'average' human body is modelled in terms of simple geometric solids of uniform activity (the standard man or other phantoms) and offers a simple method to estimate absorbed doses (148).

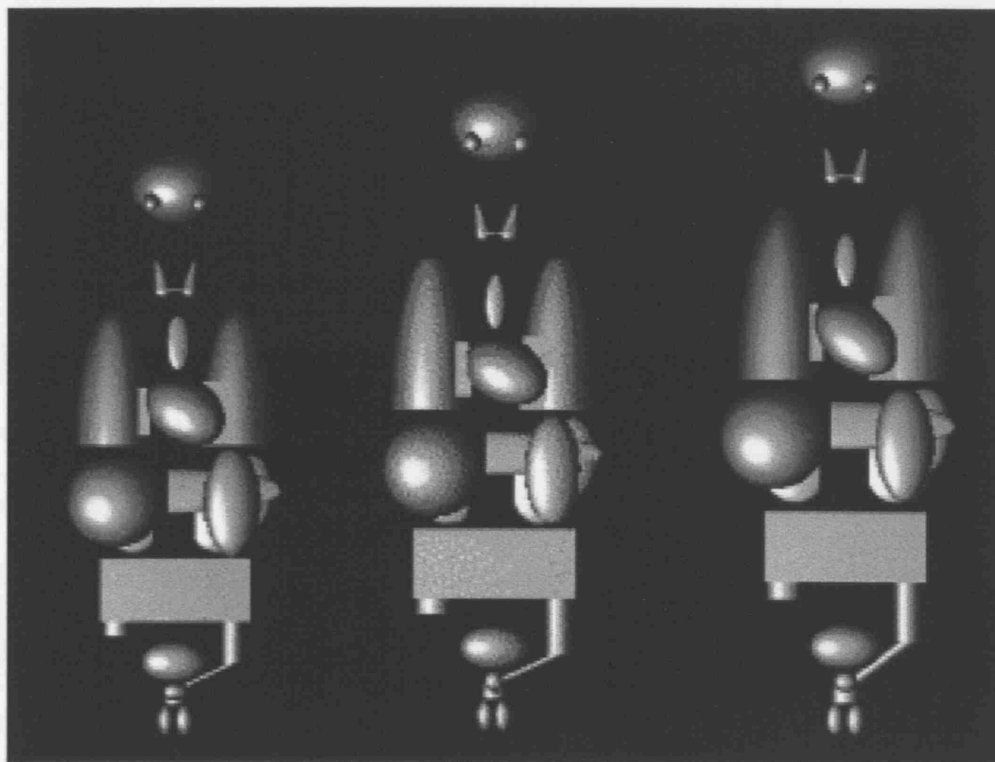
The cumulated activity in any tissue is a measure of the total number of nuclear transitions that occurred within it and dividing this by the injected activity gives the number of nuclear transitions in the organ per unit injected activity. This quotient results in a parameter with the dimensions of time, known in the MIRD schema as the 'residence time'. In the MIRD schema an organ containing activity is referred to as a 'source organ' and irradiated organs are referred to as 'target organs'. A source organ may also be a target organ for energy deposited within it, as well as from other source organs. In order to estimate the absorbed dose physical information on the mean energy of each particle emitted during decay of a particular radionuclide is also required and is obtained from nuclear data tables. The other information that is required is the fraction of the energy

from a source organ that is absorbed into itself and in other surrounding organs. This absorbed fraction depends not only upon the type and energy of the radiation but also the size, shape and composition of the source and target, the distances between source and target and the composition of the intervening tissue. In the MIRD schema, beta particles and electrons, along with photons with energies below 10-20 KeV, are considered 'non-penetrating', i.e. they are not considered an important contributor to the absorbed dose in organs outside the source. 'Penetrating' radiations on the other hand are considered to contribute significantly to radiation absorbed dose in distant organs. The specific absorbed fraction is the absorbed fraction in the target per mass of the target. The term S used in the MIRD schema refers to the mean absorbed dose in the target per unit cumulated activity and specific absorbed fractions for many pairs of organs and radionuclides have been calculated using Monte Carlo simulations in a variety of anthropomorphic phantoms. In the MIRD schema therefore, the mean absorbed dose per unit administered activity for any particular tissue is then given by the product of the residence time and the appropriate S value. It is possible by summation of the energies deposited by the various types of radiation emitted and the different source organs, to calculate the absorbed dose in target organs from the known source organs and add this to the dose that the source organs also imparts to itself, the so called self-dose, to give an estimate of the total radiation dose absorbed.

However it can immediately be appreciated that the MIRD system suffers from a number of limitations. Although the use of S values based upon population-averaged organ masses provides a convenient approach for radiation dose

estimation, the simplification of using the mass and geometry of the reference man may introduce substantial errors in radiation dose estimates as organ size varies considerably among real patients. For example in one study looking at patients with non-Hodgkin's lymphoma, CT demonstrated that 41 out of 48 patients had a spleen volume (140-2830ml, mean 380 ml) greater than that of MIRD reference man (174 ml) (149). As a result a significant error in radiation absorbed dose would occur if the MIRD S value was used. One approach to this limitation of MIRD is by determining organ S values for individual patients and a method to do this has been proposed (150). Another way in which MIRD might be improved is by having an increased number of anthropomorphic phantoms to more clearly represent anatomical variation. Clariand et al developed six novel mathematical phantoms of differing heights using the statistical analysis of anthropomorphic data gained from autopsies and are shown in Figure 1.5 (151). An investigation of the resulting S values in stomach and urinary bladder using Monte Carlo calculations demonstrated that increases in body height lead to significant differences and highlights the influence of morphology on dosimetric parameters.

Figure 1.5: Representation of internal organs of anthropomorphic mathematical models for males of height 160 cm, 170 cm and 180 cm (152)



Another simplification of the MIRD system is that it assumes homogeneous distribution of activity within tissues which is usually incorrect. The heterogeneous distribution of activity in tissues seen in RIT is important since cold spots in the tumour will lead to an increased risk of re-growth. Hot spots in organs can lead to increased normal tissue toxicity (153). The kidney for example, a frequent site of radiopharmaceutical uptake, is known to have non-uniform radioactive uptake due to its normal physiological organisation. This may lead to a considerable misrepresentation of absorbed dose if a homogeneous distribution is assumed (154). As a result, a multi-region model has been developed within the MIRD framework for kidney which requires ROI assignment of both renal cortex and renal medulla (155). Finally a further

limitation of MIRD is that there is little facility for estimating tumour dose, although an estimate of tumour self-absorbed dose can be made.

1.2.21 Clinical Application of Radiation Dosimetry in RIT

One way in which radiation dosimetry is used in RIT is for the retrospective analysis of data from the treatment of earlier patients, along with observed levels of toxicity, in order to define safe activity levels for use in future studies.

However the comparison of radiation absorbed dose with observed toxicity from one experimental group to another is difficult because of the variable biological behaviour of radiolabelled antibodies within individual patients. However in situations where retrospective dosimetry performed in phase I/II studies reveals relatively little variation in antibody distributions between patients, dosimetry using a population based dosimetry method such as the MIRD schema might be adequate to predict absorbed doses in future patients.

A clinical example of RIT that does not involve radiation dosimetry is that using the CD20 targeting antibody Zevalin. During its development, dosimetric studies have demonstrated only limited variability in urinary excretion and this, along with predictable toxicity, has allowed for safe administration based upon patient weight alone (156-160). Currently Zevalin is administered without radiation dosimetry provided the patient population meets the eligibility criteria of less than 25% bone marrow involvement with lymphoma, greater than 100,000 platelets/ mm³ at baseline and no prior bone marrow reconstitution procedure. Administered radionuclide doses for patients are calculated using

14.8 MBq/ kg as the standard dose and 11.1 MBq/ kg in patients with mild thrombocytopenia.

However, where biodistribution studies demonstrate significant inter-patient variability in activity biodistribution, a prior knowledge of individual patient biodistribution, allowing individual patient dosing might be advantageous. A high degree of variability in urinary clearance and whole body clearance has commonly been seen with ^{131}I labelled antibodies (161;162). For example in studies using ^{131}I Tositumomab, Kaminski et al reported urinary excretion rates of between 46-90% within 48 hours of antibody administration (161). As a result patient pre-treatment tracer studies are routinely used to predict normal tissue radiation exposure and determine administered activities for individual patients. Since MIRD can only provide a population estimate of absorbed dose, radiation dosimetry is performed using whole body clearance times as a surrogate for marrow absorbed dose. This has proved an effective approach since when using ^{131}I labelled antibodies it is known that emitted γ radiation is responsible for a significant proportion of the total marrow absorbed dose (92;93). In one study, individual variations in the clearance rates of antibody meant that to deliver the same total body dose, the administered activity varied from approximately 2000 MBq to 5600 MBq (163).

1.3 Clinical Radioimmunotherapy

1.3.1 Clinical Radioimmunotherapy of Non-Hodgkin's lymphoma

With their high radiosensitivity, propensity to undergo apoptosis following irradiation and abundance of well defined surface antigens, considerable success has been seen using RIT to treat the lymphomas. The potential of RIT for the treatment of B-cell NHL became apparent following the report in 1987 by DeNardo et al of the first patient with this disease treated with RIT (164). Currently a number of radiolabelled antibodies targeting several B cell antigens including CD20 (160;165-172), CD22 (173-175), and HLA class II antigens (164;176-178) are under clinical development.

Table 1.7: Examples of radiolabelled antibodies in clinical use or under development

Target Antigen	Unconjugated antibody	Generic Name (Trade name)	Corresponding radiolabelled antibody	Generic Name (Trade name)
CD20	B1	Tositumomab		¹³¹ I-Tositumomab
	2B8	Ibritumomab	IDEC- ¹¹¹ In/ ⁹⁰ Y-2B8	¹¹¹ In/ ⁹⁰ Y-Ibritumomab tiuxetan (¹¹¹ In/ ⁹⁰ Y-Zevalin)
	1F5		¹³¹ I-1F5	
	C2B8	Rituximab (Rituxan)	¹³¹ I-C2B8	
CD22	mLL2		¹³¹ I-mLL2	
	hLL2	Epratuzumab (Lymphocide)	¹³¹ I-hLL2	¹³¹ I-Epratuzumab
			¹¹¹ In/ ⁹⁰ Y-hLL2	¹¹¹ In/ ⁹⁰ Y-Epratuzumab
			¹⁸⁶ Re-hLL2	¹⁸⁶ Re-Epratuzumab
HLA-DR	Lym-1		¹³¹ I-Lym-1	
			⁶⁷ Cu-Lym-1	
			⁹⁰ Y-Lym-1	

Perhaps the greatest success with RIT so far in the treatment of B-cell NHL has been with the CD20 targeting antibodies in which considerable biological activity is already seen using unlabelled antibody. Rituximab is known to mediate a number of immune effects including ADCC, CDC, and the direct induction of apoptosis through a variety of perturbations to intracellular signalling and membrane structure (179). However, significant activity has also been seen in B-cell RIT with antibodies such as Lym-1 where naked antibody use does not lead to clinically significant anti-tumour effects.

1.3.2 Anti-CD20 antibodies

Despite the success of Rituximab in the treatment of lymphoma, half the patients with relapsed low grade lymphomas and two-thirds of patients with relapsed high grade lymphomas do not gain objective remissions with its use and moreover only a small percentage of responding patients achieve a complete remission (180;181). Radiolabelled CD20 targeting antibodies have been associated with a considerable increase in efficacy over unlabeled antibody and the two clinically available agents now represent the most effective single agents yet developed for the treatment of indolent lymphoma. The data for the clinical performance of these two different products are remarkably consistent: approximately 75% overall response rates and a 20-30% complete response rate for patients with relapsed, refractory or transformed CD20 positive indolent B-Cell NHL.

The CD20 antigen is not tumour specific and is expressed upon the surface of normal B-lymphocytes in the blood, spleen, lymph nodes and bone marrow and

also the pre-B cells in the marrow but not stem cells or progenitor cells (182). Both antibodies therefore first require the pre-infusion of unlabeled antibody as either Rituximab with the Zevalin protocol or unlabelled Tositumomab in the Bexaar regimen. Multiple pre-clinical and clinical studies have shown that pre-dosing in such a way optimises the subsequent biodistribution of the radiolabelled antibody (183;184). The unlabeled antibody binds to circulating B lymphocytes which are subsequently depleted (185;186), as well as to cells with Fc receptors throughout the reticuloendothelial system. The unlabeled antibody partially blocks binding of subsequently administered radiolabelled antibody to these sites, thereby optimising its biodistribution, increasing tumour uptake and decreasing normal organ uptake.

1.3.3 Clinical trials of ^{131}I tositumomab (Bexaar)

Kaminski et al have conducted a series of trials at the University of Michigan using ^{131}I Tositumomab (163;165;167). Patients initially receive a pre-infusion of unlabelled antibody followed by infusion of antibody trace labelled with 185-370 MBq of ^{131}I . Whole body imaging is performed three times over the following week to estimate the whole body half time and calculate the dose required for the therapeutic infusion to deliver the maximum tolerated dose of 75 cGy of whole body irradiation (usually 3330-5550 MBq). The therapeutic infusion is administered 7-14 days later. In Kaminski's single institution experience of 59 patients, a response rate of 71% was seen including 34% complete responses. The median progression free survival for all responders was 12 months, whereas it was 20.3 months for complete responders. 83% of low grade or transformed low grade lymphomas responded, compared to 41% with

de-novo intermediate grade NHL (163). Myelosuppression was the dose limiting toxicity. Non-haematological toxicities were mild comprising low grade fevers, chills, fatigue, nausea and elevated thyroid stimulating hormone levels in 8%. Vose et al have published similar results in a multi-centre phase II trial (187). In this study a 57% response rate was seen including 32% complete responses. Kaminski has also studied 76 newly diagnosed, previously untreated patients with low grade lymphomas and 97% of the patients achieved objective responses with 63% complete responses (168).

1.3.4 Clinical trials of ^{90}Y Ibritumomab Tiuxetan (Zevalin)

A similar series of trials have been conducted using the Ibritumomab antibody conjugated to ^{90}Y using the Tiuxetan chelate (Zevalin). Witzig et al performed a multicentre phase I/II study treating 51 patients with Ibritumomab tiuxetan (Zevalin) (171). In the phase I portion of the trial, patients received 100 or 250 mg/m^2 of unlabelled Rituximab followed by 7.4, 11.1 or 14.8 MBq mCi of Zevalin. The dose of unlabelled Rituximab selected for further study was 250 mg/m^2 and the maximally tolerated dose of ^{90}Y was 14.8 MBq/ Kg. On an intention to treat basis the overall response rate was 67% including 26 complete responses; 82% of indolent lymphomas responded with a 43% response rate seen in aggressive lymphoma. Haematological toxicity was dose limiting and did not correlate with dosimetric estimates performed using ^{111}In labelled antibody, the authors concluding that trace labelled infusions or dosimetry were not necessary for effective therapy. Witzig also published the first randomised clinical trial demonstrating that a radiolabelled antibody produced a higher overall and complete response rate than the corresponding naked antibody (172).

In this study 143 patients received either Rituximab (375mg weekly for 4 weeks) or ⁹⁰Y Ibritumomab tiuxetan (14.8 MBq /Kg) and the overall response rates were 56% and 80% respectively (p<0.001). The complete response rate was also higher in the group receiving RIT compared with the naked antibody group (30% vs. 16% respectively). Both regimens were well tolerated but with significantly more myelosuppression in the RIT group.

1.3.5 Anti CD22 antibodies

The CD22 antigen is broadly expressed on both normal and malignant B-cells, with a distribution comparable to that of CD20. It has demonstrated both cytoplasmic and cell surface expression, although when bound by ligand the molecule is rapidly internalised. The molecule is thought to mediate important functions in B-cell biology, including cellular adhesion and homing, as well as regulation of B cell activation (188-192). A CD22 antibody, first named EPB-2 and subsequently LL2, has subsequently been humanised (hLL2 or epratuzumab) to reduce the murine component to less than 10% and has shown activity in its unlabelled form (193).

The LL2 murine antibody targeting CD22 has a broad range of reactivity against various B cell lymphoma subtypes, as demonstrated by immunohistochemistry (194). It has been shown to effectively target the antigen and then rapidly internalise into the cell (195). Initial RIT trials using LL2 labelled with ¹³¹I have demonstrated apparent efficacy even with very low doses of radiation and this has been confirmed in a number of studies (196). Goldenberg et al have infused multiple doses of a ¹³¹I labelled anti-CD22 antibody LL2 to 21 patients with

relapsed B cell NHL and of the seventeen patients evaluable for response 4 achieved objective remissions (24%), including one complete remission (197). However given that dehalogenation of ^{131}I labelled antibodies is common following internalisation leading to loss of the radioisotope from the cell, it might seem more appropriate to label this antibody with a radiometal such as ^{90}Y to ensure maximal benefit.

1.3.6 Anti-HLA antibodies

The first antibody used in the RIT of B-cell NHL, reported by DeNardo et al, was Lym-1, a murine IgG2a monoclonal antibody that recognizes a polymorphic variant of the HLA-DR antigen expressed on malignant B cells. The antibody shows minimal cross-reactivity with normal tissues and only 5 mg of unconjugated antibody are usually given prior to therapy, except in patients with circulating malignant cells (198). Also, although Lym-1 has been shown to mediate antibody-dependent cellular cytotoxicity (ADCC) of human effector cells against a variety of malignant B-cell lines (199), naked antibody has little effect in humans. However, using the ^{131}I labelled antibody in patients with advanced, relapsed B cell malignancies leads to objective responses in approximately 50% of patients (177). Delayed haematological toxicity, especially thrombocytopenia, occurring 4-6 weeks following therapy was the main toxicity observed.

1.3.7 Clinical Radioimmunotherapy of the Common Epithelial Tumours

RIT has also been extensively investigated in the treatment of the common epithelial tumours but in comparison to the therapeutic benefits seen in NHL,

success in the treatment of these malignancies has been more limited. In part this is likely to relate to the increased resistance of these tumour types to non-surgical treatments per se, but in addition is also related to their increased radioresistance. Although during radical EBRT of lymphoma, absorbed doses of 30-50 Gy are commonly prescribed, excellent responses to low dose irradiation are common in the treatment of indolent lymphoma. In one study, 48 patients with refractory or relapsing low grade lymphoma were treated with low dose RT (4 Gy in two fractions) to a total of 135 tumour sites. The site-dependent complete response rate was 57% with an overall response rate of 81%. A long-lasting effect was seen in most patients with a 56% 2 year freedom from progression rate (200). The use of low dose local RT is also a highly effective way to give palliative treatment to disseminated tumour masses. In another recent study, local palliative RT (4 Gy in 2 fractions) was given to 22 patients with disseminated NHL or chronic lymphocytic leukaemia, with an overall response rate of 82% and a CR rate of 55% with minimal side effects (201). These doses are in contrast to those used in EBRT of solid tumours where radical doses are of the order of 60-70 Gy, i.e. nearly twice as high, and where palliative doses are usually associated with only modest antitumour effects using either 8 Gy as a single fraction or 20 Gy in five daily fractions.

During RIT the maximal absorbed tumour doses are limited by haematological toxicity secondary to bone marrow irradiation whilst antibody remains in the blood. This limits the total amount of radioactivity that can be given to patients. In addition, tumour accretion following RIT is typically only 0.001 to 0.01% of the injected activity per gram of tumour (202). As a result, tumour absorbed

doses are often considerably less than those commonly used in EBRT. Clinical radiation dosimetry performed following therapy using Zevalin in a non-myeloablative setting has estimated tumour absorbed doses of 580-6710 cGy, with a mean dose of 1700 cGy (157;158), and the use of Bexaar has been associated with mean tumour absorbed doses of only 795 cGy (187). Although such absorbed doses might prove effective for the treatment of lymphoma, the same would seem unlikely for solid tumours. In addition the low dose rates achieved by RIT might be less effective in solid tumours due to a greater dose rate effect given their lower α/β ratios. In addition solid tumours have less commonly been shown to demonstrate an 'inverse dose rate' effect, a mechanism which may be important in the efficacy of RIT.

1.3.8 Carcinoembryonic Antigen as a Target for RIT

The antigen CEA has been extensively investigated in the RIT of solid tumours and is one of the target antigens investigated in this thesis. CEA was given its name in 1965 by Gold and Freedman, who found an antigen that was present in both colonic adenocarcinoma and fetal colon, but seemed absent from normal healthy colon (203). Carcinoembryonic antigen (CEA) is a cell surface glycoprotein expressed by most colorectal tumours, and a proportion of other common epithelial malignancies such as breast, pancreatic, gastric, lung and ovarian carcinomas and is an oncofetal protein (204). It is a member of the immunoglobulin gene superfamily and is composed of a single polypeptide chain of approximately 688 amino acids and is highly glycosylated (205). It has one V-type and six C2-type immunoglobulin domains and has a molecular weight of approximately 180 kDa; the gene for CEA is on chromosome 19

(206). The molecular structure of CEA has been elucidated by small angle X-ray, neutron scattering and modelling (207). Its length has been found to be 27-33nm, and it has been shown to be arranged with its seven domains (CEA 1-7) arranged in an extended linear zigzag pattern (208;209).

CEA is expressed in normal adult tissue, with the main site being in the 'fuzzy coat' (glycocalyx) of the luminal surface of the microvilli of the columnar epithelium of the colon (210;211). It is also present to a small degree in the mucosa of the stomach, the squamous epithelial cells of the tongue, oesophagus and cervix, in sweat glands and in the epithelial cells of the prostate (212). In a normal healthy adult approximately 50-70mg of CEA is released a day into the colon (213). The normal function of CEA is not clear. Its strategic location and its structure suggest it may have a role in the maintenance of innate immunity; CEA may trap microorganisms in the glycocalyx and prevent them from invading the luminal gut cells (214). In colon cancer tumour cells lose their polarity and CEA is expressed on all surfaces (not just the luminal surface) (215;216). CEA has also been shown in tumour cell lines to act as an intercellular adhesion molecule and may have a role in the metastatic potential of malignant cells (217). It has been proposed that overproduction of CEA leads to disruption of normal intercellular adhesion forces, which may contribute to tumourgenesis by creating abnormal tissue architecture or increasing metastatic potential (218;219).

Systemically administered anti-CEA antibodies are not able to penetrate through to the luminal surface of the normal intestine due to the presence of tight

junctions between cells. As a consequence, specific tumour targeting can be achieved since normal gut epithelium is not accessible to circulating antibodies. However CEA-related antigens occur in several tissues and cross-reactivity can be a problem with non-specific anti-CEA antibodies. The most common sites of cross-reactivity are with granulocytes, monocytes, Kupffer cells, bile canaliculi, pancreatic acini and lymphocytes (220). This cross-reactivity has been shown to be as a result of shared epitopes between CEA and these tissues and it is independent of affinity (221). Most specific antibodies do not, however, have this cross-reactivity. CEA also has a secreted form, which is released into the systemic circulation. Circulating serum CEA levels are used clinically to monitor response of patients to therapy, and for early detection of tumour (222-226). Raised circulating CEA levels at the time of surgical resection have also been shown to be an independent prognostic factor for survival (227;228). The extent to which CEA is secreted into the systemic circulation varies for individual patients. A very high circulating CEA level may lead to reduced targeting efficiency of anti-CEA antibodies, as they bind to accessible CEA in the circulation, and are less available for binding to cell surface CEA in tumour areas (229). In one study a serum CEA level of > 2000 µg/L was associated with the lowest amounts of tumour anti-CEA radiolabelled antibody localisation (230). CEA expression within tumours is heterogeneous, and antibody binding throughout tumours has been shown to be non-uniform (231).

1.3.9 Antibodies targeting CEA

CEA was one of the first tumour antigens studied for radioimmunoscintigraphy and RIT. 14 trials reporting the results of ¹³¹I, ⁹⁰Y or ¹⁸⁸Re labelled anti-CEA

RIT used in a variety of clinical settings and using seven different antibodies are shown in Table 1.8. It can be seen that a number of different approaches have been taken in these studies and are illustrative of current approaches to improve the efficacy of RIT in solid tumours. One approach is to adopt techniques which reduce normal tissue exposure to radiation and which might therefore allow for dose escalation, other approaches aim to increase tumour targeting by antibody or enhance the cytotoxic effect of radiation in tumour. Finally, although most studies in RIT are performed in patients with established metastatic disease, there are sound biological and radiobiological arguments which suggest that RIT may be most effective for treating patients with smaller volume or micrometastatic disease.

Table 1.8: Published clinical studies investigating RIT directed against the CEA surface antigen

Reference	Antibody	Antibody type	No of patients	Responses	Special features
Lane et al (232)	¹³¹ I-ASB7	Murine IgG ₁ /F(ab') ₂	19	1 PR, 1 CR	Antibody fragments, repeated administration
Juweid et al (233)	¹³¹ I-NP-4	Murine F(ab') ₂	13	7 SD, 6 PD	Repeated administration/ small volume disease
Mittal et al (234)	¹³¹ I-IMMU-4	Murine IgG ₁	6	1 PR	With hyperthermia
Meredith et al (235)	¹³¹ I-COL-1/ ¹³¹ I-CC-49	Murine IgG ₁	14	4 SD	Dual Antibody with IFN α -2b
Behr et al (236)	¹³¹ I-NP-4	Murine IgG ₁	57	1 PR, 4 Mix/MinR, 7 SD	
Ychou et al (237)	¹³¹ I-F6	Murine F(ab') ₂	10	1 PR, 2 SD	Bone marrow rescue at highest doses
Juweid et al (238)	¹⁸⁸ Re-MN-14	Murine IgG ₁	11	None	Repeated administration
Behr et al (239)	¹³¹ I-MN-14	Humanised IgG ₁	12	2 PR, 5 Mix/MinR / SD	Small volume disease
Behr et al (240)	¹³¹ I-FO23C5	Murine IgG ₁	10	1 CR, 2 PR, 4 SD	Small volume disease
Hajjar et al (241)	¹³¹ I-MN-14	Humanised IgG ₁	21	None	
Behr et al (242)	¹³¹ I-MN-14	Humanised IgG ₁	30	3 PR, 8 MinR	Adjuvant RIT in 9 patient following metastatectomy
Kraeber-Bodere et al (243)	MN-14 x m734 + ¹³¹ I-hapten	Humanised IgG ₁	35	Not reported	Pre-targeted RIT
Wong et al (244)	⁹⁰ Y-T84.66	Chimeric IgG ₁	22	2 MixR, 3 SD	Repeated administration
Wong et al (245)	⁹⁰ Y-T84.66	Chimeric IgG ₁	21	11 SD, 1 MixR	Combined with 5FU

CR, complete response; PR, partial response; SD, stable disease; MixR, mixed response; MinR, minor response

1.3.10 Antibody fragments

Intact IgG antibodies, with a molecular weight of 150 kilodaltons (kDa), are large molecules, which may not penetrate well from blood into tumour. In order to increase absolute tumour uptake of antibody and also improve their intratumoural biodistribution a number of smaller antibody fragments have been

used. Applying the proteolytic enzymes pepsin and papain to whole antibodies generates a number of antibody fragments including the divalent immunoreactive fragment of 100 kDa, the $F(ab)_2$, and a monovalent immunoreactive Fab fragment of 50 kDa. As a result of their smaller size, both fragments display a more rapid and uniform tumour localisation compared to whole antibodies (153). In addition, more rapid blood clearance of these antibody fragments may also occur, improving tumour to normal tissue ratios, although rapid blood clearance also means that absolute tumour uptake may be lower than for the corresponding whole antibody (246).

Lane et al compared the biodistribution of ^{131}I labelled $F(ab')_2$ with that of its ancestor antibody, the murine IgG₁ anti-CEA monoclonal antibody A5B7 (232). In order to allow for repeated administration of antibody, Cyclosporine was also used to delay HAMA formation. In this study the antibody fragments were associated with earlier and higher tumour accumulation of antibody although there was no difference in clearance rates from normal tissues or tumour. Despite the increase in tumour absorbed dose and dose rate in the fragment arm there was no increase in efficacy noted with a 10% response rate documented in both arms of the study. However, a potential problem with using smaller antibody fragments is that they are more likely to be cleared by the kidneys than whole antibodies which are cleared via the reticuloendothelial system. This limits their usefulness for RIT since the kidney has a reduced radiation tolerance in comparison to the liver. In a further clinical study looking at the biodistribution of a humanised divalent- $F(ab')$ fragment of A5B7, high renal

uptake was demonstrated which would limit the amount of radioactivity that could be administered therapeutically (247).

1.3.11 RIT in combination with Hyperthermia

In addition to a direct radiosensitizing effect, hyperthermia in combination with RIT has been shown to increase tumour antibody uptake by increasing antigen expression and improving the blood flow and vascular permeability of tumours (248-252). Moreover RIT in combination with hyperthermia has also been shown in pre-clinical studies to improve outcomes following RIT (253;254). Mittal et al have explored the feasibility of RIT using an anti-CEA antibody in six patients with CEA-expressing colorectal cancer with liver metastasis. In this study, toxicity resulting from the hyperthermia itself included local discomfort at the catheter site, locoregional pain and one patient developed transient nausea and vomiting. In terms of response, 5 patients had a fall in CEA levels and a single partial response was observed on imaging (234).

1.3.12 RIT in combination with Interferon α

In pre-clinical tumour models the biological response modifier interferon α has been shown to enhance tumour antigen expression (255;256). This increased antigen expression has also been shown to improve antibody localisation and therapeutic outcomes following experimental RIT (257-259). Clinical studies have also found improved tumour uptake of antibody following the addition of interferon. In colorectal cancer Meredith et al have used a double antibody approach, with one antibody targeting CEA and another targeting TAG-72, both antigens overexpressed in colorectal cancer. They combined this with interferon

in a phase II clinical study (260). Although no tumour responses were seen, 4/16 patients had stable disease and 13/14 patients showed high degrees of tumour localisation leading to increased tumour absorbed doses when compared to historical controls. Side effects of interferon included fever, malaise and transient reductions in leukocytes.

1.3.13 RIT in combination with Systemic Chemotherapy

A number of chemotherapeutic agents such as 5-Fluorouracil, Cisplatin and Mitomycin C may act as radiosensitizers (261) and so enhance the effectiveness of RIT. Wong et al have examined the feasibility of using such a combined approach in the treatment of patients with metastatic colorectal cancer (262). In this study, therapy using the ^{90}Y -chimeric anti-CEA antibody T84.66 was combined with 5-FU. Although no objective responses were observed, 11 patients entering the study with progressive disease demonstrated radiological stable disease of 3-8 months duration and 1 patient demonstrated a mixed response. It was possible to administer both radiolabelled antibody and 5FU at their maximum-tolerated dose levels when given alone and RIT did not appear to increase non-haematopoietic toxicities associated with 5-FU. Only two out of 19 patients assayed developed a human anti-chimeric antibody immune response after the first cycle of therapy, which was significantly less than that observed in a previous trial evaluating treatment using the same radiolabelled antibody alone. The authors concluded that 5-FU may reduce the development of human anti-chimeric antibody response, which would permit multicycle therapies in future studies (263).

1.3.14 Increasing Tumour absorbed doses and dose rates using Myeloablative RIT

Bone marrow transplantation has allowed chemotherapy doses to be increased by 5-10 times those used conventionally (264). Since it is also toxicity to the bone marrow that limits the activity that can be safely administered to patients in RIT, this approach has also been investigated. Some of the highest response rates to RIT in lymphoma have been seen when myeloablative doses of activity have been administered (169) and high dose RIT combined with stem cell rescue has also been successfully carried out in patients with solid tumours (265). In addition to increasing tumour absorbed dose, such procedures also allow radiation to be delivered to tumours at a higher dose rate which should increase the efficiency of tumour kill through the dose rate effect. When using high dose therapy, although toxicity is generally higher than with non-myeloablative RIT, it is generally much less than that associated with high dose chemotherapy and BMT. This is thought to occur because of the absence of effects on the epithelial and endothelial barriers, although the greater morbidity and an associated mortality make this approach unsuitable for all but the fittest patients. In colorectal cancer Ychou et al have utilised marrow support in the treatment of patients with unresectable liver metastasis and this has allowed a 50% dose escalation in administered activity to be safely given (266).

1.3.15 Increasing tumour absorbed dose using pre-targeting techniques

Pretargeting techniques are another technique that may improve the therapeutic ratio of RIT and commonly involve Biotin/Avidin or Biotin/Streptavidin based systems (267;268). A number of systems have been developed but all rely upon

the rapid and efficient binding of Avidin/ Streptavidin and Biotin which ensures that radiation is targeted much more quickly and effectively than with direct antibody targeting. Such approaches have been shown to improve tumour to normal tissue ratios, both preclinically and clinically, over direct antibody targeting (268-273) but the high immunogenicity of these agents limits their use for repeated therapy (274). Kraeber-Bodere et al have used the AES pre-targeting system to treat patients with CEA expressing tumours (243). In this system a bispecific monoclonal antibody is given first and allowed to localise and then followed by the administration of a radiolabelled hapten (275;276). In their study, a chimeric bispecific antibody with a humanised anti-CEA antibody and a murine anti-hapten antibody was followed by ^{131}I labelled hapten. Although no tumour responses were seen, the authors claim that in a fully optimised system administered activities of 5.5 GBq could be safely administered and these could theoretically lead to tumour absorbed doses of 30-70 Gy.

1.3.16 Tumour Volume effects

Most of the clinical studies of RIT to date have involved patients with established metastatic disease. However in common with all radiotherapy, as tumour cell number increases, so cure probability decreases. Whereas the complete eradication of macroscopic tumour containing 10^9 viable cells might require 60-70 Gy of EBRT, less radiation will be required for smaller numbers of tumour cells. For example with histological micrometastasis where the cell number is only 10^3 cells, only one third of this dose might be required. Since absorbed doses of this order are possible with current RIT approaches this

suggests that patients with lower levels of tumour burden might be an attractive group to treat.

In addition, large tumour sizes have also been shown to have a detrimental effect upon uptake of radiolabelled antibodies per se (277;278). Looking at a variety of human tumours in pre-clinical tumour models, it has been shown that the uptake of tumour specific antibodies decreases as tumour size increases. This appears independent of the antibody class, type of tumour and target antigen (277). In a more recent study looking at mice with varying sizes of LoVo xenografts, higher antibody accumulation leading to a higher tumour absorbed dose and dose rates were also observed in smaller tumours and this translated into increased cures (278). In clinical studies an inverse correlation has also been found between tumour size and antibody uptake (279;280). Possible explanations include a reduced average vascular surface area in larger tumours compared to smaller tumours, resulting in a reduced transvascular exchange rate (281), and higher vascular permeability in smaller tumours due to lower interstitial fluid pressures (282). Further reasons why RIT might be more effective in the treatment of small volume or metastatic disease include improved homogeneity of antibody distribution in smaller tumours (283) and improved oxygenation leading to an improved response to irradiation (284).

Three trials have been reported using the humanised antibody MN-14 in patients with established metastatic gastrointestinal malignancies (240-242). Behr et al have concentrated on patients with small volume liver metastasis of colorectal cancer (less than 2 cm) whereas Hajjar et al have included patients with gross

metastatic disease of colorectal, pancreatic or gastric origin. The latter group have not observed any responses, whereas the former have reported three partial remissions and eight minor responses. Possibly due to different patient populations, however, Hajjar et al reported the MTD to be 1480 MBq/m² whereas Behr et al reported 2220 MBq/m². Behr et al were also the first group to report on the administration of adjuvant RIT using ¹³¹I MN-14 in 9 patients following complete resection of colorectal liver metastasis (242). At a follow up of 27 months only 2 patients had developed a recurrence in comparison to a historical control group treated with adjuvant chemotherapy, in which a relapse rate of 67% was noted over the same time period.

1.4 Outline Of Thesis

In terms of their favourable response to RIT it can be seen that the lymphomas are an attractive tumour type for further investigation. In this disease type, therefore, ongoing research has focused upon the identification of novel target antigens and optimisation of the clinical delivery of therapy. T-cell lymphomas and Hodgkin's disease are lymphomas in which CD20 is not expressed but which do express the interleukin 2 α component. In the second two chapters of this thesis I have therefore investigated the therapeutic potential of a novel radioiodinated antibody targeting the IL-2R α .

1.4.1 The interleukin 2 α chain as a novel target for the RIT of Lymphoma

Interleukin 2 (IL-2) acts via the interleukin 2 receptor (IL-2R) and is a potent immunomodulator which serves an important role in both the activation and maintenance of the immune response and in lymphocyte development. IL-2 is central to the control of T-cell responses and defects in the IL-2/ IL-2R receptor system are associated with severe combined immunodeficiency in humans and also one form of leukaemia (adult T cell leukaemia, caused by infection with human T-cell lymphotropic virus type I (HTLV-1)). In 1981, Waldmann and colleagues developed a murine monoclonal antibody against the receptor and called the antibody anti-tac, as it bound active T-cells only (285). This antibody is now known to recognise the alpha subunit of the receptor, a 55 kDa glycosylated type I membrane protein also known as CD25 (286). The complete IL-2R is now known to comprise three transmembrane protein chains α (CD25), β (CD122) and γ (CD132) (287;288).

The α subunit gene is located on chromosome 10 in humans (289) and the protein product of this gene is a 251 amino acid chain. This has a long extracellular domain of 219 amino acids, responsible for binding IL-2, a 19 amino acid hydrophobic region thought to be the transmembrane domain and a C-terminal intracellular domain of only 13 amino acids. The latter is thought to be too short to act as an important site for signal transduction and lacks any known consensus sequences for intra-cellular signalling. The IL-2R α chain is selectively expressed upon activated T lymphocytes unlike the more ubiquitous expression of the β and γ chains. The β subunit is a 70-75 kDa protein of 525 amino acids with its gene locus located on chromosome 22 (290). It has a 214 amino acid extracellular domain, a 25 amino acid transmembrane region and an intracellular domain of 286 amino acid residues. It is constitutively expressed on resting lymphocytes, monocytes, macrophages and neutrophils and is up-regulated upon T-cell activation. The most recent chain to be identified is the γ chain, this again is a type I membrane protein and is 347 amino acids in size giving a molecular weight of 64 kDa. The genetic locus is on the X chromosome (291). It has a long intracellular domain that is vital for IL-2 signalling and is constitutively expressed on lymphocytes, monocytes and neutrophils.

IL-2 will bind to individual receptors but with low affinity. The α subunit on its own has the lowest affinity; the $\beta\gamma$ complex has an intermediate affinity whereas all three receptor chains are required for high affinity binding and is shown in Table 1.9.

Table 1.9: Receptor binding affinities of the IL-2R receptor components

Receptor complex	Binding affinity (K_d M)
α chain	10^{-8}
β and γ chains	10^{-9}
α, β, γ chains	10^{-11}

1.4.2 CHT-25- A chimeric antibody targeting the IL-2R α chain

There are currently at least two antibodies targeting the IL-2R α including the chimeric antibody CHT-25 (Basiliximab) and a humanised form of Waldmanns' anti- tac antibody (Daclizumab). These antibodies have been used in a variety of clinical settings including the treatment of malignant disease, autoimmune disease and the suppression of organ transplant rejection. Both antibodies bind with high affinity to the IL-2R α and are proposed to mediate their effects by blockade of IL-2 binding and possibly also by leading to ADCC and CDC (292). CHT-25 is derived from a mouse monoclonal antibody produced at the Royal Free Hospital (RFT5 γ 2 α) and is the antibody studied in this thesis. The murine variable regions have been retained and the constant regions of the heavy and light chains have been replaced by human constant regions which consist of the heavy chain of IgG1 and the kappa light chain. It is produced in vitro by continuous culture fermentation of a murine-myeloma cell line transfected with plasmid-borne recombinant gene constructs coding for murine variable regions and human constant regions. CHT-25 binds to the 55kD α chain of the IL-2 receptor with an affinity approximating IL-2 itself and in vitro studies have demonstrated that 1 μ g/ml of CHT-25 completely inhibited binding of radiolabelled IL-2 to its receptor. CHT-25 without any radioactive label has already been used in human studies looking at the prophylaxis and treatment of

acute rejection episodes in kidney transplant recipients and is currently licensed for the prevention of acute cellular rejection in renal allografts (64;293). In transplant patients the mean terminal half-life has been estimated at 13.1 days (7-23 days) and CHT-25 has demonstrated low immunogenicity even following multiple administrations.

1.4.3 IL-2R α antibodies and HTLV associated adult T-cell leukaemia/ lymphoma

The first malignancy to be targeted using IL-2R α targeting antibodies was HTLV associated adult T-cell leukaemia/ lymphoma. This is a disease caused by the malignant proliferation of mature T-cells that infiltrate the lungs, skin and central nervous system. The disease is endemic in Japan, the Caribbean and central Africa, reflecting the distribution of the causal agent-a type C retrovirus called human T-lymphotrophic virus type I and is associated with hypercalcaemia, an immunodeficiency state and an aggressive clinical course (292). HTLV-1 is known to integrate into the T-cell genome and leads to the production of a 42 kDa protein termed tax that is essential for viral replication. Tax up-regulates the transcription of IL-2, IL-2R α and NF κ B genes by binding to sequences in the promoter regions of these genes which in turn leads to the constitutive expression of IL-2R α at up to 5-10 times those seen in the maximally stimulated normal T-cell. Tumour cells therefore express the IL-2R α at a very high density of 10^3 to 10^4 sites per cell (294). The additional secretion of IL-2 is thought, at least in the early stages of the disease, to form an autocrine feedback loop important for stimulating cellular division, though as disease progression occurs, chromosomal abnormalities and genetic mutations

allow IL-2 independent cell division. These observations led Thomas Waldman and his group to propose that by interrupting the cytokine-mediated growth of the malignant cells using anti-tac, apoptotic cell death of tumour cells may occur due to IL-2 deprivation, a novel therapeutic strategy for a disease in which the response to conventional therapies is poor. In an initial study using naked murine anti-tac and looking at 19 patients, a mean total dose of 225 mg and a mean number of five antibody infusions was administered (estimating that 40-100 mg of anti-tac was necessary to saturate the IL-2R α upon tumour cells). Responses were seen in 7 patients (1 mixed response, 4 partial responses and 2 complete responses) (295). Since murine antibodies are unable to lead to host immune responses it was assumed that tumour cell kill was due to blockade of the growth factor receptor, so called cytokine depletion. Failure of response was thought to be due to cytokine independence seen in the later stages of the disease. Following the success of naked anti-CD25 antibody in the treatment of HTLV T cell leukaemia/ lymphoma, Waldmann et al subsequently utilised a radiolabelled antibody in an attempt to increase response rates. In this study 18 patients with advanced disease were enrolled and treated with ^{90}Y labelled antibody to a dose of 185-555 MBq. 16 patients were available for evaluation and of these 7 developed a partial remission with 2 complete remissions. Toxicity was restricted to delayed granulocytopenia and/or thrombocytopenia in 12/18 patients (296).

1.4.4 Abnormal Expression of IL-2R α in other tumour types

In addition to its abnormal expression in HTLV associated adult T-cell leukaemia/ lymphoma, lower levels of IL-2R α expression are also detectable

upon malignant cells from approximately 50% of patients with B-cell chronic lymphocytic leukaemia (297;298), up to 100% of cells of anaplastic large-cell lymphoma (299), 33-55% of B-cell NHL (300;301), 20-50% of peripheral T-cell lymphomas (302;303), 40-50% of cutaneous T-cell lymphoma (304;305), 75-90% of patients with Hodgkin's disease (306;307) and 80% of patients with hairy cell leukaemia (308). This combined with its limited physiological expression (identified in only 5% of the circulating lymphocyte population), makes it an attractive target for RIT in these tumour types and forms the basis of the second and third chapters of this thesis.

1.4.5 Improving the therapeutic ratio of RIT in the treatment of Colorectal Cancer

In contrast to lymphoma, RIT of the common epithelial malignancies has been less successful and presents different problems. An important factor in the relative failure of RIT compared to the lymphomas is their relatively increased radioresistance leading to an inadequate therapeutic ratio. As has already been discussed, a number of approaches have already been taken to improve the efficacy of RIT in patients with CEA-expressing tumours. In the fourth chapter of this thesis I have continued my study of RIT using the well investigated monoclonal anti-CEA antibody A5B7 to treat CEA-expressing colorectal cancer in a pre-clinical tumour model. In particular I have investigated the use of dose-fractionated RIT as an alternative technique to improve the response to therapy.

1.4.6 A5B7 antibody- A murine antibody targeting CEA

A5B7 is an IgG1 murine antibody to CEA (309;310) and an F(ab')₂ fragment of A5B7 with a molecular weight of 100 kDa has also been derived from whole A5B7 by enzymatic digestion with pepsin (311). A5B7 whole IgG and its F(ab')₂ fragment have been used extensively both preclinically and in clinical trials of radioimmunotherapy, radioimmunoscintigraphy and radioimmunoguided surgery (232;246;312;313). As A5B7 is a murine antibody it is immunogenic in humans, and without the concurrent administration of immunosuppressive drugs, only one administration is usually possible before human anti-mouse antibodies form (314). In an attempt to improve response rates to RIT using A5B7, it is currently being investigated in a phase I/II study in combination with the antivascular drug Combretastatin A-4 3-O- Phosphate. This is a tubulin binding agent that in tumour models leads to haemorrhagic necrosis within the central region of tumours (315;316), whilst sparing the well vascularised regions of the tumour surface which are generally supplied by relatively normal vessels from surrounding tissue (317;318). Used as a single agent, Combretastatin has a limited effect on tumour cure as re-growth is invariably seen from cells in viable areas at the tumour rim. However when used in combination with RIT in a human colon cancer xenograft model, it has been able to convert tumour growth delay into tumour eradication (319;320) . Proposed mechanisms for this effect include efficient killing of radiosensitive tumour cells in the well perfused areas of the tumour by RIT, entrapment of RIT within tumour as a result of tumour vessel collapse, combined with destruction of more hypoxic radioresistant tumour cells at the tumour centre by Combretastatin.

1.4.7 Dose fractionation as a means to improve the therapeutic ratio of RIT

Dose fractionation is established practice in EBRT and has been shown to lead to an increase in the therapeutic ratio by decreasing late normal tissue toxicity allowing for significant dose escalation. In addition, during a typical six week fractionated course, tumour shrinkage leads to a process of revascularisation which is thought to overcome hypoxic radioresistance (99). With the advent of antibodies of reduced immunogenicity it has become possible to give repeated administrations of radiolabelled antibodies and fractionation of RIT has been proposed as another method to improve the therapeutic ratio of treatment.

However although there are sound radiobiological arguments for the fractionation of the high dose rate irradiation typical of EBRT, there are less in favour of its adoption in RIT. Increasing the fractionation of radiotherapy generally leads to reduced tumour kill due to the dose rate effect, an observation that will be particularly noted in the more radioresistant solid tumours. In addition due to prolongation of the overall treatment time therapy will also become less effective due to increased repopulation. Improving the therapeutic ratio by fractionation therefore relies upon the ability to dose escalate leading to cell kill over and above that lost through the mechanisms described above as a result of normal tissue sparing. However RIT is already highly fractionated and as a result significant further reductions in normal tissue toxicity as a result of fractionation would seem unlikely. In addition unlike EBRT where late responding normal tissues are dose limiting, with RIT the dose limiting organ is bone marrow, which is an acute responding tissue and therefore less influenced

by dose rate. Marrow sparing as a result of fractionation might be achieved through prolongation of the overall treatment time however, which in the absence of marrow ablation would allow a greater opportunity for repopulation during therapy (140;321).

Pre-Clinical studies supporting fractionation of RIT

Despite these radiobiological arguments a number of pre-clinical studies have suggested that fractionated RIT may offer a therapeutic advantage however (322-327). In the study by Schlom et al using the whole monoclonal antibody B72.3 in a murine xenograft model, and where the same total administered activity was divided into 2 or 3 fractions, enhanced anti-tumour effects were seen as a result of fractionation (326). Another study looking at fractionation of the equivalent administered activity using the single chain Fv CC49 similarly revealed a significant advantage to tumour control in the fractionated group compared to the single fraction arm (325). Buchsbaum et al using the CC49 whole antibody also observed a significant reduction in tumour growth when the same total activity was given as two fractions rather than a single fraction, but only when given three days apart. Increasing the inter-fraction interval to 7 days resulted in a marked loss of efficacy with no tumour regressions seen (323). A more recent study looking at fractionated intra-peritoneal low dose RIT in a murine gastric cancer tumour model using a ^{213}Bi conjugated monoclonal antibody has similarly demonstrated a therapeutic advantage to the administration of double injections of antibody at a seven day interval in comparison to a single injection of twice the activity (328).

A number of possible explanations for the increased efficacy of fractionated RIT have been proposed. It has been argued that the anticipated reduction in effectiveness as a result of reduced dose rate might be less important in RIT if a significant 'inverse dose rate' effect were to occur. In addition dose rate effects might also be offset if a significant low dose hyperradiosensitivity effect were seen. Furthermore it has also been suggested that the longer treatment times associated with fractionated RIT might actually be advantageous to cell kill by allowing more opportunity for effective tumour shrinkage to occur. This in turn could lead to improved blood flow and a reduction in tumour interstitial pressure and so improve the access of subsequently delivered RIT, in addition to allowing for tumour re-oxygenation to occur (215;259). Pre-clinical studies have also demonstrated that fractionated RIT improves the delivery of antibody to the well vascularised actively growing areas of tumour (324;329). Using autoradiography to assess antibody location within tumour, it has been observed that whereas with single administration of antibody activity fell rapidly at the well vascularised and actively dividing tumour rim, repeated administrations tended to maintain activity for longer in this area and so enhanced cell kill.

A concern that has been raised regarding the use of fractionated RIT however is that changes in tumour vascular physiology have been observed following fractionated RIT, which might reduce its effectiveness. Blood endothelial cells are amongst the most radiosensitive of the mesenchymal tissues (330) and pre-clinical studies by Buchsbaum et al have demonstrated decreases in blood vessel density and reductions in blood vessel diameter following RIT which in turn have reduced the accretion of subsequent doses of antibody (282;331). However

in a more recent comparative study by the same group, looking at a wide range of tumour xenografts treated with RIT, some tumours demonstrated unchanged or increased vascular permeability suggesting that the vascular effects of RIT might be variable (332). However the data outlined above would seem to suggest that fractionation of a given activity of RIT might be associated with an increase in efficacy; even in the absence of dose escalation as a result of the additional radiobiological factors outlined above.

With regard to toxicity, three studies have looked specifically at haematological toxicity (322;326;327). Beaumier et al demonstrated a reduction in white blood cell toxicity as a result of fractionation (322) whilst Schlom et al demonstrated that marrow aplasia could be avoided (326). The most comprehensive study of the effects of fractionated RIT on haematological toxicity has been undertaken in non-tumour bearing animals (327). In this study using beagle dogs, haematological toxicity was assessed by daily blood counts and in addition early marrow progenitor function was assessed with colony forming unit assays for granulocytes and macrophages (CFU_{gm}). Single injections were associated with earlier and more severe blood cytopenias (thrombocytopenia and granulocytopenia) but also with lower bone marrow CFU_{gm} counts than fractionated RIT.

Previous work in this department using a fragment of ¹³¹I-A5B7 has demonstrated reduced efficacy as a result of dose fractionation however. The therapeutic efficacy of intact and F(ab')₂ fragments of ¹³¹I labelled A5B7 were compared in an established LS174T colonic xenograft model in nude mice. A

single IV dose of either 18.5 MBq intact or 37 MBq F(ab')₂ fragments significantly delayed tumour growth, and increased survival time to the same extent. However fractionating the 37 MBq dose of F(ab')₂ into three doses of 12.2 MBq, given on days 1, 3 and 5, significantly reduced the therapeutic effect of treatment (246). Other preclinical studies have shown that the precise details of the fractionation regime are important in determining outcome however, and therefore a more detailed investigation of fractionation using a wider variety of fractionation regimes is necessary to exclude a benefit to tumour kill by fractionation. Furthermore no assessment of haematological toxicity was made in this study, either as acute blood counts or by assessment of marrow progenitor cells. As a consequence it remains unclear whether fractionation is of benefit to the therapeutic ratio in this tumour model system.

1.5 Thesis Hypotheses

Three major hypotheses will be tested in this thesis:

- a. That radioimmunotherapy with ^{131}I -CHT-25 will be an effective treatment modality of low immunogenicity for patients with IL-2R expressing lymphomas.

The IL-2R α is an attractive target for RIT given its limited expression in normal tissues combined with abnormal expression in a number of lymphomas. The hypothesis that ^{131}I -CHT-25 is an effective agent for RIT of patients with antigen- expressing lymphomas will be examined in Chapters 2 and 3. In Chapter 2, response and toxicity data from a dose escalation study utilising ^{131}I -CHT-25 for the first time in patients with antigen-expressing lymphomas will be presented.

- b. That the adoption of individual patient dosing based upon tracer studies may improve the therapeutic ratio following RIT using ^{131}I -CHT-25 in patients with relapsed/ refractory IL-2R expressing lymphomas.

The development of most systemic therapies for use in cancer is based upon dose escalation studies which aim to define a single maximum tolerated dose for a given therapeutic agent. However often there are significant differences in biodistribution between patients which during RIT may easily be measured when utilising ^{131}I as the therapeutic radionuclide. In Chapter 3 this hypothesis will be examined by correlating biodistribution data and MIRD population

based dosimetry with observed levels of haematological toxicity data from the phase I/II clinical trial of ^{131}I -CHT-25.

c. That fractionation of Radioimmunotherapy using ^{131}I -A5B7 in a colorectal tumour xenograft model will lead to an improvement in the therapeutic ratio of treatment.

Dose fractionation of RIT has been proposed as a means to improve the therapeutic ratio of RIT. In Chapter 4 this hypothesis will be examined with a presentation of the effects of fractionation upon therapeutic efficacy as well as both systemic and haematological toxicity in a murine xenograft model.

2. A Phase I/II Trial of Radioimmunotherapy with intravenous ¹³¹Iodine-labelled Chimeric (Mouse/Human) Monoclonal Antibody (CHT-25) to the IL-2 Receptor in patients with Refractory IL-2 Receptor Expressing Lymphomas: Presentation of Responses and Toxicity data from 9 patients.

2.1 Introduction

2.1.1 Background

Lymphomas represent an attractive target for radioimmunotherapy given their inherent radiosensitivity and the presence of well defined surface antigens on B and T lymphocytes. A number of B cell antigens have been successfully targeted leading to clinical responses, these include HLA-DR and CD22 (177;178;196). However the greatest success to date has been achieved by targeting CD20 which is expressed upon more than 90% of B-cell lymphomas. Radiolabelled CD20 targeting antibodies have proved an effective therapy for lymphoma in both myeloablative and non-myeloablative settings and two are currently licensed for clinical use (163;165;167;169;171;172). Given the success of RIT in B-cell NHL a number of other cell surface antigens are currently being explored as potential therapeutic targets for RIT including the interleukin 2 receptor alpha subunit (also referred to as CD25). Whole antibodies targeting CD25 have lead to successful therapy of HTLV-1 associated adult T-cell leukaemia/ lymphoma both as naked antibody and as a ⁹⁰Y labelled radioimmunoconjugate (296;333). Furthermore a recombinant immunotoxin containing a single chain Fv fragment of the same anti-Tac antibody fused to a truncated form of the bacterial toxin pseudomonas exotoxin has also

demonstrated clinical evidence of efficacy in the treatment of CD25-expressing haematological malignancies (81).

2.1.2 IL-2R α as a target for RIT

The IL-2R comprises three transmembrane protein chains α (CD25), β (CD122) and γ (CD132) (287;288). The α chain is an attractive target antigen for RIT given that it has limited physiological expression upon activated B and T lymphocytes and monocytes (334) but is also expressed upon a variety of haematological malignancies. IL-2R α is consistently expressed at very high density upon the cells of HTLV-1 associated adult T-cell leukaemia/ lymphoma (10^3 to 10^4 sites per cell) (335), but lower levels are detectable upon malignant cells from approximately 50% of patients with B-cell chronic lymphocytic leukaemia (336;337); up to 100% of cells of anaplastic large-cell lymphoma (338); 33-55% of B-cell NHL (339;340); 20-50% of peripheral T-cell lymphomas (341;342); 40-50% of cutaneous T-cell lymphoma (343;344); 75-90% of patients with Hodgkin's disease (345;346) and 80% of patients with hairy cell leukaemia (347).

2.1.3 Current treatment strategies in Hodgkin's disease and T- cell NHL

Current treatment approaches in Hodgkin's disease and T- cell NHL comprise a combination of systemic chemotherapy and external beam radiotherapy.

Hodgkin's disease

Hodgkin's disease is a comparatively uncommon disease, although in young adults it is one of the most common malignancies. In England and Wales, for

instance, it accounts for 0.5% of cancers overall (excluding non-melanoma skin cancer), but at ages 15-24 it accounts for 1 in 6 cancers (348). Advances in the treatment of Hodgkin's disease in the 1960's and 1970's mean that mortality from Hodgkin's disease has fallen by over two-thirds since the 1960's (349) and today greater than 80% of patients are cured (350). Traditionally, early stage disease was treated with radiotherapy alone but more recently combined modality approaches comprising chemotherapy followed by limited field radiation are commonly used. Such approaches have proven effective therapy for early stage Hodgkin's disease and most patients are cured (351;352). In advanced stage Hodgkin's disease the introduction of combination chemotherapy in the 1970's revolutionised treatment for this group of patients (353). Current Anthracycline based regimes are associated with complete response rates and 5 year failure free survival rates of 82% and 61% respectively (354). However about a third of patients will still eventually relapse, although a proportion of these may still be cured with salvage therapy comprising either further conventional chemotherapy or high dose chemotherapy (355;356).

T-Cell Lymphoma

T-cell lymphomas are a heterogeneous group which include primary cutaneous T-cell lymphomas, adult T-cell leukaemia or lymphoma and peripheral T-cell lymphoma. T-cell NHL are rare in Europe and the United States where they comprise about 15-20% of aggressive lymphomas but are more common in Asia where they are frequently associated with infection with the human T-lymphotrophic virus (357). Mycosis fungoides and the Sezary syndrome (the

cutaneous T-cell lymphomas) for the most part represent low grade malignant lymphoid proliferations and have an indolent clinical course. A wide range of approaches have been used to treat these tumours although none appear to significantly affect survival (358). In contrast the peripheral T-cell lymphomas (PTLC) and adult T-cell leukaemia/ lymphoma are aggressive malignancies (357).

Peripheral T-Cell Lymphoma

Peripheral T-cell lymphomas (PTCL) make up the majority of T-cell NHL in adults in the West. Excluding cutaneous and HTLV-1 associated T-cell lymphomas, approximately 10% of all NHL are PTCL (359). In a large series encompassing 288 cases and comparing to high grade B-cell NHL, PTCL were found to have a higher incidence of disseminated disease (78% vs. 58%), B symptoms (57% vs. 40%) and bone marrow involvement (31% vs. 17%) (360). No standard therapy has been established to treat this type of lymphoma, but when therapeutic regimens used for high grade B-cell lymphoma are applied, the T-cell phenotype usually (361-363), but not in all cases (364;365), confers a poor prognosis. In one series the complete response rates to therapy for B-cell NHL c.f. T-cell NHL was less (63% vs. 54%) as were the 5 year overall survival rates (53% vs. 41%). This study concluded that even accounting for the presence of adverse prognostic factors at diagnosis, T-cell phenotype was an independent negative prognostic factor (366). Similarly to B-cell NHL, high dose chemotherapy is used to salvage patients with failure to achieve a complete response with first line chemotherapy or who subsequently relapse following first line chemotherapy and may cure a third of these patients (367;368).

Adult T-Cell Leukaemia/ lymphoma

This is a disease caused by the malignant proliferation of mature T-cells that infiltrate the lungs, skin and central nervous system. The disease is endemic in Japan, the Caribbean and central Africa, reflecting the distribution of the causal agent- a type C retrovirus called human T-lymphotrophic virus type I and is associated with hypercalcaemia, an immunodeficiency state and an aggressive clinical course (292). Most patients are treated with combination chemotherapy but the results following a variety of approaches have been disappointing with no conventional treatment programme able to induce long term disease free survival. A total of 854 patients with HTLV-1 associated T-cell leukaemia/lymphoma diagnosed between 1983 and 1987 were analysed for prognostic factors and survival following combination chemotherapy by the lymphoma study group (369). The median survival time and projected two and four year survival rates for all patients were 10 months, 28% and 12% respectively (370).

2.1.4 Therapy of HTLV associated T-cell leukaemia with IL-2R targeting antibodies.

The first malignancy to be targeted using an IL-2R targeting antibody was HTLV associated adult T-Cell leukaemia/ lymphoma. HTLV-1 is known to integrate into the T-cell genome and leads to the production of a 42 kDa protein termed 'tax' that is essential for viral replication. Tax up-regulates the transcription of IL-2, IL-2R α and NF κ B genes by binding to sequences in the promoter regions of these genes which in turn leads to the constitutive

expression of IL-2R α at up to 5-10 times that seen in the maximally stimulated normal T-cell. The additional secretion of IL-2 is thought, at least in the early stages of the disease, to form an autocrine feedback loop important for stimulating cellular division, although as disease progression occurs, chromosomal abnormalities and genetic mutations allow IL-2 independent cell division. These observations led Waldman et al to propose that by interrupting the cytokine-mediated growth of the malignant cells using an IL-2R α blocking antibody (called anti-tac), apoptotic cell death of tumour cells may occur due to IL-2 deprivation, a novel therapeutic strategy for this disease (371). In an initial study using murine anti-tac and looking at 19 patients, a mean total dose of 225 mg and a mean number of five antibody infusions were administered (estimating that 40-100 mg of anti-tac was necessary to saturate the IL-2R α upon tumour cells). In this study responses were seen in 7 patients and included 1 mixed response, 4 partial responses and 2 complete responses (295) with failure of response thought to be due to cytokine independence commonly seen in the later stages of the disease. Following the success of naked murine anti-CD25 antibody the same group subsequently utilised radiolabelled antibody in an attempt to further increase response rates. In this study, 18 patients with advanced disease were enrolled and treated with ⁹⁰Y labelled antibody to a dose of 185-555 MBq. 16 patients were available for evaluation and of these 7 developed a partial remission with 2 complete remissions. Toxicity was restricted to delayed neutropenia and/or thrombocytopenia in 12/18 patients although six patients developed anti-murine antibodies which limited the ability to give repeated administrations (296).

2.1.5 CHT-25-A chimeric antibody targeting the IL-2R α subunit

CHT-25 is a chimeric monoclonal antibody derived from a mouse monoclonal antibody produced at the Royal Free Hospital (RFT5 γ 2 α). The murine variable regions have been retained and the constant regions of the heavy and light chains have been replaced by human constant regions which consist of the heavy chain of IgG1 and the kappa light chain. It is produced *in vitro* by continuous culture fermentation of a murine-myeloma cell line transfected with plasmid-borne recombinant gene constructs coding for murine variable regions and human constant regions. CHT-25 binds to the 55 kD α chain of the IL-2 receptor with high affinity ($K_a = 1 \times 10^{10} \text{ M}^{-1}$) (372).

CHT-25 without any radioactive label has already been used in human studies looking at the prophylaxis and treatment of acute rejection episodes in kidney transplant recipients. The specific high affinity binding of CHT-25 to IL-2R- α competitively inhibits IL-2 mediated activation of lymphocytes, a critical pathway in the cellular immune response involved in allograft rejection. CHT-25 is currently licensed for the prevention of acute cellular rejection in renal allografts (64;293). In transplant patients the mean terminal half-life has been estimated at 13.1 days (7-23 days) and CHT-25 has demonstrated low immunogenicity even following multiple administrations.

2.1.6 High dose RIT with Autologous Bone Marrow Transplantation/ Peripheral Blood Stem Cell transplantation

High dose combination chemotherapy with or without local or extended field radiotherapy, supported by autologous bone marrow transplantation (AMBT), is

now an accepted treatment modality in patients with refractory or relapsed Hodgkin's disease and non-Hodgkin's lymphoma (373;374). In RIT the dose limiting toxicity is also haematological toxicity and autologous stem cell support has successfully allowed safe dose escalation in this setting (169;375). Peripheral Blood Stem Cell Transplantation (PBSCT), a more recent advance, offers some advantages over traditional ABMT including a simpler collection procedure which can be achieved in an out-patient setting and a shorter time to engraftment (376)

2.1.7 IL-2R as a target for RIT in lymphoma

In this study therefore we hypothesised that the selective physiological expression of IL-2R α and its abnormal expression in lymphoma would make it an attractive target for RIT of antigen expressing lymphomas. In this phase I/II dose escalation study we have radiolabelled the chimeric antibody CHT-25 with ^{131}I and used this to treat patients with IL-2R expressing lymphomas, with the primary intention of defining a safe administered activity for future studies but also to look for preliminary evidence of efficacy. An important difference between the use of radiolabelled CHT-25 and the currently available radiolabelled CD20 antibodies is its chimeric design which, as outlined in Chapter 1, is often associated with altered pharmacokinetics, in particular a prolonged circulating half-life. In the development of the commercially available CD20 targeting antibodies the use of longer lived variants has not been favoured because of concerns about normal tissue toxicity. An important question regarding the use of chimeric antibody to deliver RIT is whether this prolonged exposure of normal tissues, and in particular bone marrow, will have

a detrimental effect upon the therapeutic ratio. With regard to this it is likely that the activity leading to dose limiting marrow toxicity with chimeric antibodies is likely to be less than that of a murine counterpart. This has been suggested by a study investigating the biodistribution and kinetics of ^{131}I labelled Rituximab in which the dose factor for the whole body was about twice as high for Rituximab compared to that reported for ^{131}I -Tositumomab due to the prolonged circulating half-life of the former (377). In a comparison of two studies both using the tracer dose method of Wahl et al (378) to deliver 0.75 Gy whole body dose, doses for ^{131}I Tositumomab have been reported as ranging from 2 GBq to 5.6 GBq (163), whereas for ^{131}I -Rituximab these ranged from 1.7-4.3 GBq (379). We have also anticipated that in addition the reduced mouse protein content of this chimeric antibody should be associated with a reduced incidence of neutralising antibody formation in comparison to a whole murine antibody, allowing for repeated administration of antibody.

For patients treated at an injected activity of 1480 MBq/m² of ^{131}I and above, autologous bone marrow harvest or peripheral blood stem cell harvest has been carried out in all patients. This has allowed for dose escalation to continue with the safety of a 'back up' rescue should marrow recovery not occur. In this chapter I present data relating to the toxicity and efficacy of ^{131}I -CHT-25 in the nine patients treated so far in the study.

2.2 Trial Overview

The trial has been conducted as a single centre, open label, non-comparative dose- escalation phase I/II trial. In the first phase ^{131}I -CHT-25 has been administered at a fixed dose of 10 mg and the amount of ^{131}I conjugated with this dose escalated to define the maximum tolerated dose. In the second phase patients were treated at the MTD in order to investigate efficacy. The trial incorporates conventional phase I trial measures including toxicity and pharmacokinetics. The pharmacokinetic data collected during the study has been utilised to perform radiation dosimetry and is presented in Chapter 3.

2.2.1 Clinical trial objectives

The primary trial objectives have been to determine the dose limiting toxicity (DLT) and maximum tolerated dose (MTD) of ^{131}I -CHT-25. Additional primary objectives included the determination of its pharmacokinetic profile in blood and urine and an investigation of its localisation, biodistribution and radiation dosimetry. Secondary trial objectives included the documentation of any preliminary evidence of efficacy during the dose escalation phase and an assessment of immunogenicity.

Tumour response has been assessed by clinical and radiological methods, using WHO criteria, and also by measuring changes in tumour glucose uptake by fluorodeoxyglucose positron emission tomography (FDG PET) scans.

2.2.2 Definition of Dose limiting toxicity (DLT)

Toxicity has been assessed according to the National Cancer Institute Common Toxicity Criteria. A DLT was defined as almost certainly or probably drug related and could occur in any cycle of treatment and included:

1. Grade IV febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection)
2. \geq Grade 2 neurotoxicity
3. \geq Grade 3 toxicity to organs other than the bone marrow excluding:-
 - grade 3 nausea
 - grade 3 or 4 vomiting in patients who have not received optimal treatment with anti-emetics
 - grade 3 or 4 diarrhoea in patients who have not received optimal treatment with anti-diarrhoeals
4. Drug related death

In this study, grade IV haematological toxicity did not constitute a DLT as ABMT or PBSCT was performed when neutrophils fell to $0.2 \times 10^9/L$ or less for 2 consecutive days using previously stored bone marrow or stem cells.

2.2.3 Definition of Maximum Tolerated Dose (MTD)

The MTD for this trial was defined as a dose level below that at which a single drug related death was observed and/ or where more than 30% (two patients out of up to a maximum of six patients) of the patient population suffered dose limiting toxicity due to the drug. Following determination of the MTD the

second phase of the trial will commence. In this phase it is required that a total of 14 patients, all evaluable for response are treated at the MTD. If there are no responses (partial or complete), the trial will be terminated. If a confirmed response is seen in one or more patients, 11 additional patients will be recruited. This will ensure that the standard error of the observed response rate will be less than or equal to 0.1. This scheme ensures that if the drug is active in 20% or more patients, the chance of erroneously rejecting the drug after the first 14 patients is 0.044. The advantage of a two stage scheme is that it allows early rejection of an ineffective drug.

Patients with refractory IL-2R expressing lymphoma were entered into this trial and it was expected that the trial would commence recruitment in March 2000 and have a duration of 30 months. In fact the first patient was treated in December 2002 with a total of nine patients treated to July 2004 which are reported here.

2.2.4 Patient population and selection

Patients with refractory histologically proven IL-2R expressing lymphomas were eligible for entry into the trial. Patients were all treated at the Royal Free Hospital and consisted of both internal and external referrals and met the following inclusion and exclusion criteria.

Inclusion criteria

1. Refractory, histologically proven lymphoma i.e. patients who have been treated with at least a first or second line conventional chemotherapy which may include ABMT/ PBSCT and have subsequently relapsed. Where there is no established, conventional therapy or the patient refuses conventional therapy the patient may be entered for the study after fully informed consent
2. Lymphoma immunohistochemically shown to express IL-2R on at least 50% of the HD or NHL cells from previous diagnostic test. Expression of CD30 in at least 50% of HD cells was an acceptable surrogate for CD25 if previous formalin-fixed material was used. CD30 expression was not an acceptable surrogate for other NHL.
3. Bidimensional measurable disease either clinically or radiologically by X-ray, CT, US or MRI scan
4. Age ≥ 18 years.
5. Life expectancy ≥ 3 months.
6. WHO performance status of 0, 1 or 2
7. No anti-lymphoma treatment in the previous 4 weeks (if on steroids a stable dosage over the same period is required).
8. At dose levels of up to 740 MBq/m^2 of ^{131}I , with clinicians discretion, a stem cell harvest or bone marrow harvest for ABMT or PBSCT was not required. For doses of 1480 MBq/m^2 of ^{131}I and above a stem cell harvest (minimum requirement is $2 \times 10^6/\text{L}$ CD34 positive cells) or bone marrow harvest (minimum requirement is $1 \times 10^6/\text{L}$ CD34 positive cells) for ABMT or PBSCT was required
9. Haematological and biochemical indices:

- i) Absolute neutrophil count of $\geq 1.5 \times 10^9$ /litre.
- ii) Haemoglobin ≥ 10 g/dl.
- iii) Platelet count $\geq 100 \times 10^9$ /litre.
- iv) Plasma creatinine ≤ 150 μ mol/l or EDTA or urine creatinine clearance ≥ 50 ml/min.
- v) Plasma bilirubin ≤ 30 μ mol/l.
- vi) ALT /AST ≤ 2 x upper limit of normal (5x upper limit of normal in the presence of liver metastases).
- vii) INR ≤ 1.5 .
- viii) Thyroid function either normal or if the patient has a known thyroid abnormality, it must be stable on treatment.

10. Cardiac function – ejection fraction of greater than 50% measured by MUGA or Echocardiogram (ECHO scan) at rest.

11. Provide written informed consent and are capable of co-operating with treatment and follow-up.

Exclusion Criteria:

- 1. Patients who have received radiotherapy (except for palliative reasons), endocrine therapy, immunotherapy or chemotherapy within four weeks (six weeks for nitrosureas and Mitomycin-C) prior to treatment.
- 2. $> 25\%$ bone marrow involvement by lymphoma
- 3. Pregnant or lactating women, or women of child bearing potential in whom pregnancy cannot be excluded. Patients of childbearing/ child fathering potential not using adequate, medically approved contraception 4 weeks before, during the study and for six months afterwards.

4. Patients who are a high medical risk because of non-malignant systemic disease, as well as those with active uncontrolled infection.
5. Known to be serologically positive for Hepatitis B, Hepatitis C or Human Immunodeficiency Virus (HIV).
6. Severe respiratory disease.
7. Any other conditions, which in the Investigator's opinion would not make the patient a good candidate for the clinical trial.
8. Positive HAMA to CHT-25.

2.2.5 Selection of the Phase I Starting Dose and Schedule

CHT-25 was administered at a fixed dose of 10mg. This dose has been shown in phase I studies to be safe and well tolerated (293). It has been calculated that this dose provided sufficient antibody to allow adequate targeting to the IL-2R receptor of lymphoma cells. The dose of 370 MBq of ^{131}I was chosen as a starting dose based upon dosimetric calculations which predicted that it would be a dose which would not produce significant myelosuppression or non-haematological side effects. These calculations were based on dosimetry experience during a radioimmunotherapy/ radioimmunoscintigraphy trial using a chimeric B72.3 anti-CEA antibody administered at the Royal Free Hospital (65). It was assumed that the distribution of CHT-25 into tissue not expressing IL-2R should be the same as the distribution of the B72.3 anti CEA antibody into tissues not expressing CEA.

2.2.6 Dosing schedule/ Treatment Schedule

Treatment consisted of the administration of ^{131}I on Day 1 and follow up for at least 8 weeks or until drug related toxicity resolved to baseline or another anti-cancer treatment was introduced. Repeated dosing of ^{131}I CHT-25 was allowed as early as 4 weeks following therapy provided the criteria for repeat dosing were fulfilled (Section 2.3.12). Patients were entered progressively in groups of three and received treatment with escalating doses of ^{131}I -CHT-25 as outlined in Table 2.1.

Table 2.1: Dose escalation protocol for dose escalation phase of CHT-25 study

No of Patients	Treatment	Dose increased by
3	10 mg of CHT-25 labelled with 370 MBq/m ² of ^{131}I	-
3	10 mg of CHT-25 labelled with 740 MBq/m ² of ^{131}I .	x 2
3	10 mg of CHT-25 labelled with 1480 MBq/m ² of ^{131}I .	x 2
3	10 mg of CHT-25 labelled with 2220 MBq/m ² of ^{131}I .	x 1.5
3	10 mg of CHT-25 labelled with 2960 MBq/m ² of ^{131}I	x 1.3

A time interval of at least 7 days between treating each new patient at the same dose level was required. Also a time interval of at least 4 weeks was required between the first patient being treated on a previous dose level and entry of a new patient at the next dose level, to take into consideration experience gained at the lower dose level.

2.2.7 Expansion of dose level(s).

If one out of three patients experienced a DLT, up to three further patients could be recruited at that dose level. If only one out of six patients at a particular dose level experienced a DLT, dose escalation was allowed to continue unless a drug related death was observed. Dose escalation was stopped when two patients out of a

maximum of six, at one dose level, experienced a DLT. When two patients at a particular dose level experience a DLT or one patient experienced a drug related death, treatment would de-escalate to the previous dose level or at a lower dose level to define MTD. Six patients could be treated at this dose in order to establish the MTD.

2.2.8 Repeat dosing/ intra-patient dose escalation

Dose escalation or a repeat treatment at the same dose level was allowed in the same patient after a minimum of 4 weeks, provided:

1. There was evidence of adequate tumour localisation of CHT-25 antibody, as assessed by gamma camera imaging (tumour uptake of radiolabelled antibody \geq 3% injected dose/kg using analysis of SPECT images).
2. Patient experienced no DLT at current dose level.
3. Rescue with PBSCT or ABMT was not required at the current dose level.
4. Pre study eligibility criteria remain fulfilled (other than haematological).
5. Resolution of any drug related toxicities that were \geq grade 2 (other than haematological).

Patients who did receive further treatment at a higher dose level were included as one of the three patients at the new dose level. A patient could be escalated to the next dose level for their repeat doses even if no new patients had been treated at that dose level.

Treatment continued as per protocol unless there was progressive disease or the patient either asked to be withdrawn or was experiencing unacceptable toxicity.

All patients that received treatment were evaluated for toxicity and response.

Concomitant medication was administered as medically indicated and details noted. Radiotherapy could be given concomitantly for the control of bone pain or as indicated, however these irradiated lesions would not be evaluable for response. The patient was not allowed to receive other anti-cancer therapy or investigational drugs while on trial. Steroids could continue to be administered if the dose was stable over the 4 weeks prior to the trial. Patients whose dose of steroid was increased during the trial were not evaluable for disease response and would be removed from the study. Thyroid blockade was administered prior to and following therapy in accordance with the trial flow sheet to reduce the risk of thyroid ablation.

2.2.9 Pharmaceutical data

CHT-25 was provided by Novartis for the purposes of the study and was supplied as 10 mg vials lyophilised for reconstitution. CHT-25 was stored in a secure limited access storage area in the Royal Free Hospital Pharmacy at 2-8°C. ^{131}I was ordered through the Department of Medical Physics and supplied by Amersham as a sodium iodide solution in standard vials and transported in a lead pot. ^{131}I was stored at room temperature in the radioactivity storage area of the Department of Medical Physics until it was used for labelling.

2.2.10 Method of Reconstitution and Radiolabeling

Reconstitution was performed during the labelling process by a suitably qualified radiopharmacist in the hospital Nuclear Medicine Department. Radiolabelling was performed using the N-Bromo-succinamide/ L-tyrosine technique (380). The reconstitution and radiolabelling procedure comprised the following steps:

1. Reagents: -

CHT-25 (10 mg), sterile water, 1M phosphate, dose of Sodium ^{131}I Iodine (MBq), working solution of N-Bromosuccinimide at 1 mg/ml, working solution of L-Tyrosine at 2 mg/ml, 80% Methanol and 100% Ethanol.

2. Procedure: -

NB. All procedures were carried out aseptically in a sterile cabinet.

1. Each reagent was well mixed to ensure homogeneity.
2. The correct volume of each of the following was drawn up into separate sterile syringes: concentrated antibody, phosphate buffer, N-Bromosuccinimide and L-Tyrosine leaving an air gap between plunger and agent.
3. All materials were aseptically transferred to the radioactive "Hot Lab".
4. The rubber seal on the top of the pot containing Na ^{131}I was wiped with a steret swab and a spinal needle inserted.
5. 0.1 ml of 1M phosphate buffer was added.
6. 10 mg/ml of concentrated antibody was added.
7. 70 μl of working N-Bromosuccinimide solution (at 1mg/ml) was added.
8. 45 seconds was allowed to pass before adding 0.1 ml of working L-Tyrosine solution (at 2 mg/ml).
9. 10 μl of the product was removed and added to 450 μl of sterile water (1/50 dilution) for Thin Layer Chromatography (to determine percentage incorporation of ^{131}I onto protein) and High Performance

Liquid Chromatography (HPLC) analysis). Immuno-reactivity of labelled product was confirmed by FACS.

The radiolabelled ^{131}I -CHT-25 antibody was subsequently transported to the ward according to the regulations of the Medical Physics department. Administration of ^{131}I -CHT-25 was carried out with full resuscitation facilities available in case of any severe anaphylactic reactions to antibody and was as follows:

- Two medium bore intravenous cannulas were inserted in the patient, preferably one in each arm.
- Automatic monitoring of pulse, blood pressure and oxygen concentration was set up.
- A continuous infusion of 0.9% saline was commenced at approximately 150 mls/ hour into one cannula. This continued throughout the time when the radiolabelled antibody was administered and continued afterwards for at least 1 hour.
- The other cannula was used for administration of the radiolabelled antibody. The syringe containing the radiolabelled antibody was placed behind lead and connected to the patient via a 1m infusion line and three-way tap. The pump was initially set to deliver the antibody at 6 mg/hour. If after monitoring for ten minutes there were no significant side effects or greater than 20% change in vital signs, the infusion rate was increased to 12 mg/hour. A further doubling of administration rate was possible after 10 minutes if the patient remained symptom free to a maximum of 24 mg/hour. The total time for the infusion of antibody was approximately 40 minutes.

- When the infusion was completed a 20 ml syringe containing saline was attached to the infusion pump and given at the same rate for 5 minutes to flush the line.
- The radioactive syringe, infusion line, three way tap and intravenous cannula were subsequently removed and stored in the radioactive materials transfer trolley for later disposal.

Before administering the study drug, the exact dosage was always double-checked by a second suitably qualified person and documented. Vein extravasation and accidental spillages were dealt with according to hospital policy. The hospital policy was guided by a radioprotection representative, who was present at all times during administration of ^{131}I -CHT-25.

2.3 Investigation Schedule

2.3.1 Pre-treatment Evaluations

The haematological and biochemical indices stated in the inclusion criteria required to assess eligibility were performed within one week prior to the patient starting treatment. Baseline evaluations to assess tumour/ disease could be performed within four weeks prior to the start of treatment but were preferably performed within two weeks. In order to be evaluable for response, radiological baseline assessments occurred at least four weeks after previous treatment for disease, unless the assessment of the disease clearly indicated progression following previous treatment. Clinical measurements to assess tumour/ disease were done within one week prior to the patient starting treatment. Blood was

taken for HAMA (5ml blood in a plain clotted tube) within 12 weeks of starting treatment.

2.3.2 Evaluations during the course of the trial

These are outlined in the trial schedule of events shown in Table 2.

Where blood and urine tests were requested the following tests were performed:

Haematology	Haemoglobin, white cell count, neutrophils, lymphocytes and platelets
Biochemistry	Sodium, potassium, urea, creatinine, urate, total protein, bilirubin, AST, ALT, Alkaline phosphatase, glucose, GGT
Clotting	PT, APPT, INR
Thyroid function tests	Plasma T4 and TSH
Urinalysis	Blood, protein, glucose and pH

2.3.3 Patient Follow up

This continued for 9 weeks following therapy and any drug related toxicity that had not resolved to baseline after this period was observed until resolution or another anti-cancer therapy was commenced.

Table 2.2: Schedule of Events for CHT-25 study

	Pre-treatment	Baseline		Treatment													Follow up							
Parameter	Within 2 Weeks	Day -3	Day -1	Day 1 Wk1	Day +2	Day +3	Day +4	Day +5 to +7	Day +8 Wk2	Day +11 Wk2	Day +15 Wk3	Day +18 Wk3	Day +22 Wk4	Day +25 Wk5	Day +29 Wk5	Day +36 Wk6	Day +43 Wk7	Day +50 Wk8	Day +53	Day +57	Follow Up			
Informed consent	X																							
Registration	(within 1 week) X																							
Medical History	X																							
Physical examination	X		X	X					X		X		X		X	X	X	X		X				
Tumour assessment (MRI, CT, X-ray, U/S or Clinical measurement, if applicable)	X														X					X	X			
FDG PET Scan	X													X					X					
Chest X-ray	X																							
ECG	X														X					X				
Potassium iodide admin		X 100 mg tds	X 100 mg tds	X 50 mg bd	X 50 mg bd	X 50 mg bd	X 50 mg bd	X 50 mg bd	X 50 mg bd	X 50 mg bd														
Inpatient stay			X	X	X	X	X	(X)	(X)	(X)														
Bone marrow aspirate and Trepphine	X														(X)					(X)				
WHO	X		X						X		X		X		X	X	X	X		X				
Height, Weight, BSA	X																							

Parameter	Pre-treatment	Baseline		Treatment												Follow up					Follow Up
		Day -3	Day -1	Day 1	Day +2	Day +3	Day +4	Day +5 to +7	Day +8 Wk2	Day +11 Wk2	Day +15 Wk3	Day +18 Wk3	Day +22 Wk4	Day +25 Wk5	Day +29 Wk5	Day +36 Wk6	Day +43 Wk7	Day +50 Wk8	Day +53	Day +57	
24hr creatinine clearance	X															X				X	
Haematology	(within 1 week) X		X		X	X	X	(X)	X	X	X	X	X	X		X	X	X		X	
Biochemistry	(within 1 week) X		X		X	X	X	(X)	X	X	X	X	X	X		X	X	X		X	
Clotting	(within 1 week) X		X								X				X		X			X	
Thyroid function tests	X														X					X	
5mls of clotted blood for immunology	X								X		X		X		X	X	X	X		X	
10 mls of heparinised blood for T lymphocytes			X						X		X		X		X	X	X	X		X	
Urinalysis			X						X		X		X								
CHT-25 Drug infusion				X 12:00																	
Symptom History			X	X	X	X	X	(X)	X	(X)	X		X		X	X	X	X		X	
Vital Signs				X	X	X	X	X													
PK analysis				X	X	X	X	(X)	(X)	(X)											
Gamma camera imaging				X +4 hr	X	X	X	(X)	(X)	(X)	(X)										
ECHO/MUGA scan	X														X					X	
24 hr urine collection				X	X	X	X	(X)	(X)	(X)											

• After day +28 treatment may be repeated in the same patient at the same or next dose level (start from day -3)

• Bone marrow aspirate/trephine is optional

- 5mL of blood is taken into a clotted tube pre treatment and weekly for at least 8 weeks after treatment for :
 - CHT-25 levels (measured by RIA)
 - antibodies to CHT-25 – HAMA (measured by ELISA and FACS analysis)
- 10mL of blood is taken into a heparinised blood tube for measurement of levels of circulating T lymphocytes which express IL-2R (measured by FACS analysis)
- Vital Signs (temp, pulse, bp, resp. rate) – taken before antibody infusion, after infusion and at hourly intervals for 2 hr then 6 hourly during inpatient hospitalisation.
- PK samples taken after completion of antibody infusion 2 mins, 2 hrs, 4 hrs, 6 hrs.

2.3.4 Assessment of Safety

The NCI Common Toxicity Criteria (CTC) version 2.0 was used to grade adverse events. The worst grade in a cycle for that particular event was documented. If the AE resolved completely or resolved to baseline and then worsened again this was recorded as a separate AE event.

2.3.5 Assessment of Efficacy

A thorough clinical and radiological evaluation of malignancy was performed in all patients prior to starting the trial. Baseline investigations included a physical examination, chest x-ray and as indicated CT scans, ultrasound, MRI or bone scans. All radiological baseline assessments were performed within four weeks prior to starting treatment but preferably within 2 weeks. Tumour assessments were repeated every 4 weeks or more frequently when clinically indicated. Lesions defined at baseline were followed by the same methods throughout the study. In order to be evaluable for response, radiological baseline assessments needed to occur at least four weeks after previous treatment for disease, unless the assessment of the disease clearly indicated progression following previous treatment. Response was assessed using standard WHO response criteria based upon change in the maximal bidimensional diameters of lesions.

2.3.6 FDG-PET imaging

Response assessment was also assessed using fluorodeoxyglucose positron emission tomography (FDG PET) scans. Patients were imaged on an ADAC Vertex Plus Dual Head Co-incidence Camera.

Patient preparation

Patients were asked to fast for 5 hours prior to injection of [F-18] FDG. [F-18] FDG was acquired from a commercial offsite cyclotron. 100 MBq of [F-18] FDG was administered, and scans commenced at 90 minutes post injection. Patients were rested after injection. Blood glucose levels were recorded by dextrostix and 3 mL of blood was also removed for blood glucose level assessment. The patients then rested for 1 hour prior to the scan being performed.

[F-18] FDG PET scanning protocol

The scan performed comprised of a whole-body scan and included where possible chest, abdomen and pelvis with the scanning time usually 1-2 hours. The scan consisted of both a transmission and emission scan to allow full attenuation correction. Transmission scans were performed with Caesium-137 point sources. The set-up position of the patient was recorded and each subsequent scan utilised the same patient set-up procedure. Reconstruction was performed using standard ADAC iterative reconstruction protocols, with correction for scatter and attenuation algorithms. Analysis consisted of visual comparative assessment and quantitative analysis using a semi-automated region growing programme.

Semi-Automated programme for assessing changes in [F-18] FDG uptake in tumour as a result of therapy (381;382).

Assessing changes in [F-18] FDG uptake may be approached in a number of ways. Visual analysis involves comparison of scans taken at different time points and depends upon the change in tumour compared to background tissue.

However this is a subjective method and may therefore be problematic in relation to reproducibility, and reliability depending upon operator experience and fatigue. One semi-quantitative technique that has been adopted to overcome this is the use of the standardised uptake value (SUV) and has been widely adopted for quantification of intensity of [F-18] FDG uptake in tumours. The SUV is a value derived from the mean tumour radiotracer concentration (MBq/L), which is then corrected for injected activity. A region of interest (ROI) is placed within an area of tumour and an SUV obtained pre and post therapy. A reduction in SUV indicates a response to treatment and an increase indicates progression. SUV analysis is performed on static PET images, which are acquired after [F-18] uptake into cells is complete, usually assumed to be 60 minutes post injection. However SUV has a number of limitations including an absence of agreement on the size or placement of ROI, or whether mean or maximum counts per voxel should be recorded. In addition SUV accounts for tumour intensity alone and does not represent any changes in tumour volume or mass that may occur as a result of therapy. In this study therefore use has been made of a semi-quantitative technique that allows for both changes in tumour morphology and intensity of metabolic activity to be assessed. In this technique an automated iterative adaptive threshold based region programme is used to delineate tumour volumes in an objective and reproducible way. The operator identifies the tumour and then places a seed point anywhere within this. The operator then defines a threshold value and the programme will grow the tumour region contiguously from the seed point to include any voxels with counts per voxel above the selected threshold. The mean is recalculated after each iteration and the threshold value reapplied, with the process continued until no further voxels can be drawn. To ensure reproducibility, once the final volume has been

defined, iteration stops and the region is re-grown from the maximal count/voxel outwards. In such a way it is ensured that wherever the seed-point is placed within the tumour the same region will always be grown. Quantification of the tumour uptake then takes account of both tumour intensity and size and the total VOI counts has been shown to be the most reliable index with which to compare tumour response to therapy.

2.3.7 Administration

This trial has been conducted in compliance with the EU Clinical Trials Directive, the principles of ICH GCP and the Declaration of Helsinki as amended in South Africa (1996). This trial has been conducted under a Doctors' and Dentists' exemption (DDX) and was sponsored and monitored by Cancer Research UK (formerly The CRC).

2.4 Results

2.4.1 Patient Demographics

Since the trial opened, nine patients with measurable, relapsed/ refractory IL-2R expressing lymphomas have been enrolled with the first patient treated in December 2001 and the last treatment administered in July 2004. The patient demographics are summarised in Table 2.3.

There have been seven males and two females with a median age of 36. Patients had been heavily pre-treated with six of the nine having previously undergone a high dose procedure with stem cell rescue.

Table 2.3: Patient demographics for CHT-25 study. This reveals a heavily pre-treated population group, with most of the patients having a diagnosis of Hodgkin's disease.

<u>Patient ID</u>	<u>Gender</u>	<u>Age</u>	<u>Histology</u>	<u>Previous therapy</u>	<u>Sites of Disease at entry to study</u>
01	Female	34	ALCL Hodgkin's like lymphoma	CHLVPP/ PABLOE, CHOP,BEAM + PBSCT, RT to right SCF	Internal mammary. left PA , cervical, axillary LN, anterior chest wall, skin
02	Female	70	High Grade B cell NHL	CHOP + IT MTX, RT to right axilla and neck	Anterior abdominal wall, right kidney, axillary, PA and mesenteric LN
03	Male	36	Hodgkin's disease	PABLOE, CHLVPP/ PABLOE, EPIC, BEAM + PBSCT	Mediastinum,PA + axillary LN, Liver, Spleen
04	Male	34	Hodgkin's disease	ABVD, LOPP/ EVAP, IVE, BEAM + PBSCT, RT to mediastinum	SCF, mediastinum, lung
05	Male	62	Peripheral T-cell lymphoma	CHOP, IVE, Cyclophosphamide, Beam+ PBSCT	Mediastinum
06	Male	33	Hodgkin's disease	ABVD, CMOPP, CHLVPP, Splenectomy, IVE, mini BEAM + PBSCT	Mediastinum, cervical, inguinal, axillary, abdominal and hilar LN
07	Male	42	Hodgkin's disease	ABVD, CHLVPP, BEAM + PBSCT, Stamford V, Chlorambucil, Gemcitabine, Lomustine	Lung, spleen, cervical, axillary LN
08	Male	62	HTLV associated adult T-cell lymphoma	CHOP + IT MTX, High dose MTX	Cervical, axillary, inguinal, PA LN, Skin
09	Male	27	Hodgkin's disease	ABVD, ESHAP, mini BEAM, mediastinal RT	PA, axillary, abdominal LN, lung

2.4.2 Baseline Haematological Function

Prior to each therapy, gradable haematological toxicity was seen in many of the patients including anaemia (14/18), low total WCC (2/18), lymphopenia (12/18), neutropenia (1/18) and thrombocytopenia (6/18) and are shown in Table 2.4. In patients undergoing repeated therapy there was a higher incidence of gradable toxicity prior to therapy; anaemia (9/9), thrombocytopenia (8/9), lymphopenia (8/9) and neutropenia (1/9).

Table 2.4: Incidence of CTC gradable haematological toxicity *prior* to therapy with ¹³¹I-CHT-25

Patient ID and therapy no	Treatment number	Haemoglobin	Platelets	Total WCC	Lymphocytes	Neutrophils
1	1	1	0	0	0	0
2	1	0	0	0	2	0
3	1	1	0	0	2	0
3	2	1	0	0	2	0
4	1	1	0	0	2	0
4	2	1	1	0	2	0
4	3	1	3	0	2	0
4	4	1	2	0	2	0
5	1	1	0	0	0	0
5	2	1	1	1	0	0
6	1	1	0	0	0	0
7	1	1	0	0	0	0
7	2	2	1	0	2	0
7	3	2	3	2	2	2
8	1	2	0	0	0	0
9	1	1	0	0	2	0
9	2	1	0	0	2	0
9	3	1	0	0	2	0

2.4.3 Administered Activity

A total of 18 antibody infusions have been performed with five patients undergoing repeated therapies and the maximum number of antibody administrations received by any one patient being four. The maximal total

administered activity for any single patient was 6660 MBq/m². No serious acute adverse events were seen with infusion of antibody; fatigue, nausea and fever were the only significant side effects seen. All were CTC grade 1 in severity. Fatigue was documented following three administrations, pyrexia following two administrations and nausea following a single treatment. Five patients received multiple therapies as outlined in Table 2.5.

Table 2.5: Administered activity and cumulated administered activity in MBq/m². Actual administered activities are shown in brackets.

Patient ID	Treatment no. 1	Treatment no. 2	Treatment no. 3	Treatment no.4	Cumulative Activity
01	370 (663)				370 (663)
02	370 (573)				370 (573)
03	370 (725)	740 (1377)			1110 (2102)
04	740 (1380)	1480 (1868)	2220 (3395)	370 (554)	4810 (7197)
05	740 (1286)	1480 (2220)			2220 (3506)
06	740 (1104)				740 (1104)
07	1480 (2397)	2220 (2560)	2960 (4553)		6660 (10507)
08	740 (1093)				740 (1093)
09	740 (1105)	1480 (2104)	2220 (3239)		4440 (6448)

2.4.4 Haematological Toxicity

The prime toxicity associated with therapy has been delayed blood cytopenias and the typical pattern seen is shown in Figure 2.1. The relationship of observed toxicity to administered activity is shown in Table 2.6. Generally toxicity has been mild and reversible at the lower activity levels with grade 4 toxicities (except for lymphopenia) confined to activities at or above 1480 MBq/m² administered activity except in a single patient. This patient (04) had already received three antibody infusions and received therapy at the lowest activity level as a palliative procedure despite a significant number of gradable haematological cytopenias at baseline. Three patients treated at higher activity

levels required platelet support, and stem cell infusion was required in a single patient receiving the highest total administered activity following a period of prolonged neutropenia. This patient subsequently died following failure of engraftment which was complicated by *Pneumocystis carinii* pneumonia. Following this episode the highest activity level (2960 MBq/m²) was removed from the trial protocol and subsequent patients all commenced therapy at 740 MBq/m² to increase safety. Persistent haematological toxicity has primarily been confined to patients receiving multiple therapies and higher total administered activity.

Figure 2.1: Typical pattern of haematological toxicity following RIT with ¹³¹I-CHT-25. An early drop in blood lymphocytes is seen, reflecting interphase death in this cell population, followed by a dose dependent fall in platelets and less commonly neutrophils and total WCC at around one month with slow recovery over the next few weeks depending upon administered activity.

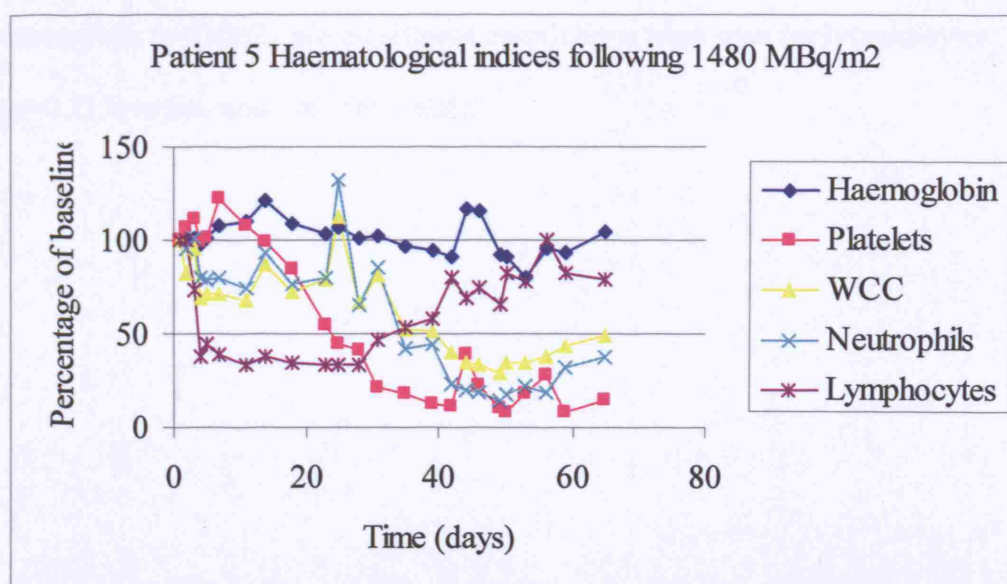
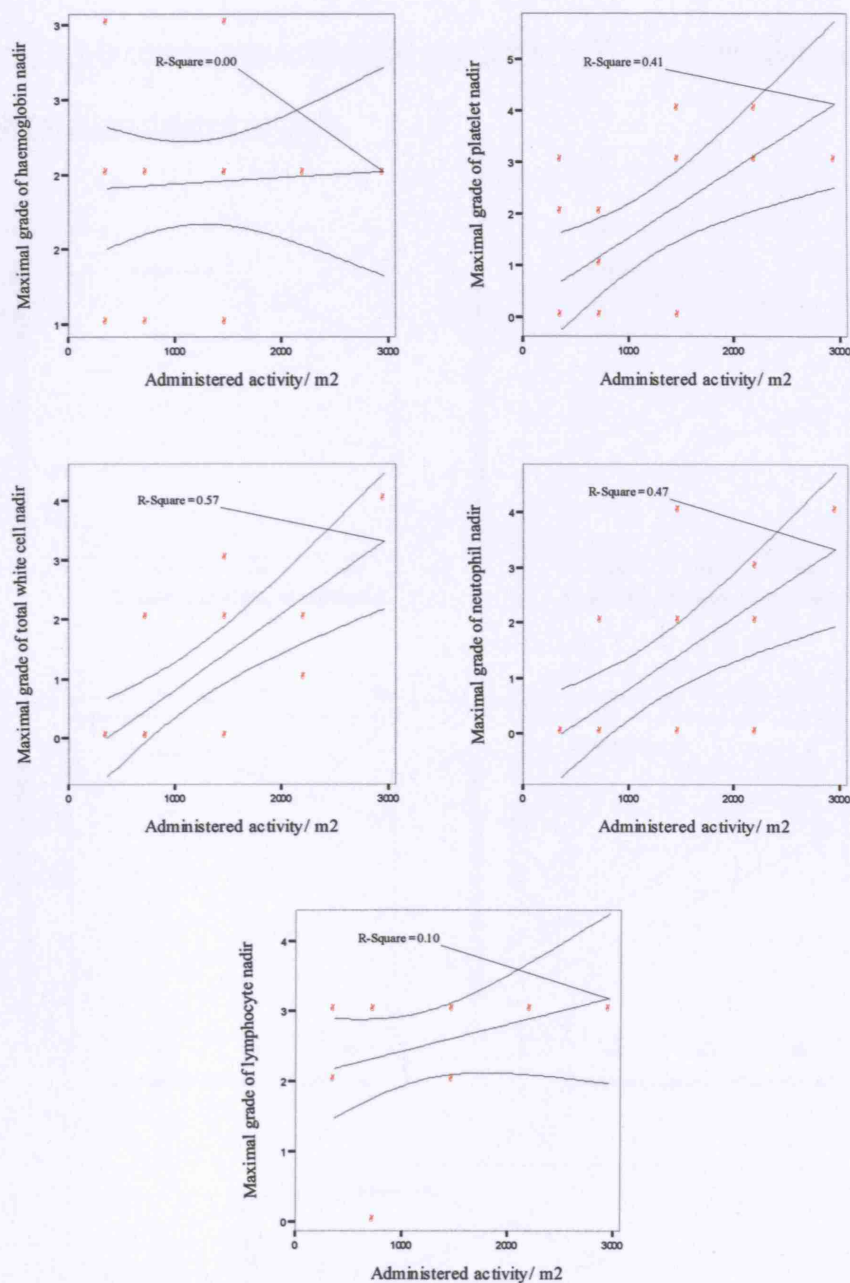


Table 2.6: Maximum CTC nadir grades of haematological toxicity in relation to administered activity.

Patient no	Dose level (MBq/m²)	Haemoglobin	Platelets	Total WCC	Lymphocytes	Neutrophils
1	370	2	0	0	1	0
2	370	2	2	0	3	0
3	370	1	0	0	3	0
4	370	3	3	0	2	0
3	740	1	0	0	3	0
4	740	2	1	0	3	0
5	740	2	2	2	3	2
6	740	2	0	0	0	0
8	740	2	0	0	0	0
9	740	2	0	0	3	0
4	1480	2	3	0	3	2
5	1480	2	4	3	3	4
7	1480	2	3	2	2	3
9	1480	1	0	0	3	0
4	2220	4	4	0	3	2
7	2220	2	4	2	3	3
9	2220	2	3	1	3	4
7	2960	2	4	3	4	4

Pearson correlation coefficient analysis was performed to look for any correlation between administered activity and toxicity and is shown in Figure 2.2. This revealed significant correlations between administered activity and nadir grade for platelets ($p=0.004$), total white cell count ($p<0.001$) and neutrophils ($p<0.002$). No significant correlations were seen for lymphocytes ($p=0.213$) or haemoglobin ($p=0.805$).

Figure 2.2: Maximum haematological toxicity by CTC grade in relation to administered activity with associated linear regression line + 95% mean predicted interval.



Since a number of patients underwent repeated therapy, an additional analysis of observed haematological toxicity in relation to cumulative administered activity was also performed using linear regression and is shown in Figure 2.3.

Pearson's correlation coefficient analysis revealed significant association for platelets ($p=0.002$), total WCC ($p=0.013$) and neutrophils ($p=0.032$) but not for haemoglobin ($p=0.206$) or lymphocytes ($p=0.317$)

Figure 2.3: Maximum haematological toxicity by CTC grade in relation to cumulated administered activity.

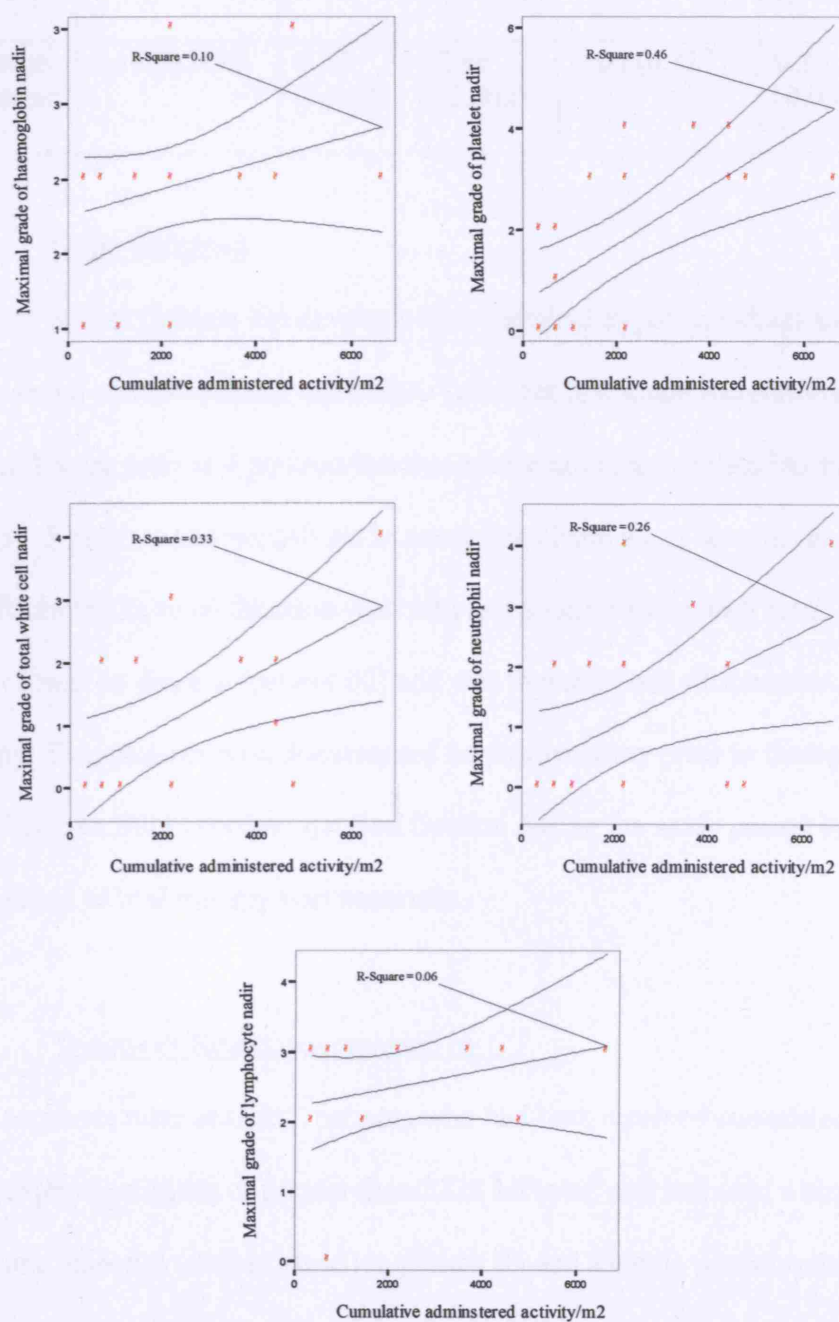


Table 2.7: Correlation between observed CTC grade of haematological toxicity and either single or cumulated administered activity using Pearson's correlation coefficient with associated p values.

	Pearson Correlation r^2 value (p-value)				
	Haemoglobin	Total WCC	Platelets	Lymphocytes	Neutrophils
Single administered activity	0.00 (0.805)	0.57 (<0.001)	0.41 (0.004)	0.10 (0.213)	0.47 (0.002)
Cumulative administered activity	0.10 (0.206)	0.33 (0.013)	0.46 (0.002)	0.06 (0.317)	0.26 (0.032)

2.4.5 Other toxicities

A single patient (patient 04) developed biochemical hypothyroidism and was commenced on replacement thyroxine. Transient low grade increases in GGT and ALT were seen in 4 patients but these were not clearly related to the trial therapy. 5 patients had reductions in creatinine clearance at baseline and a significant fall in renal function was seen in a single patient with renal involvement by disease (patient 02) and was therefore not attributed to the trial therapy. Two patients with documented cardiomyopathy prior to therapy experienced a fall in cardiac ejection fraction during the study period but again the relation to trial therapy was uncertain.

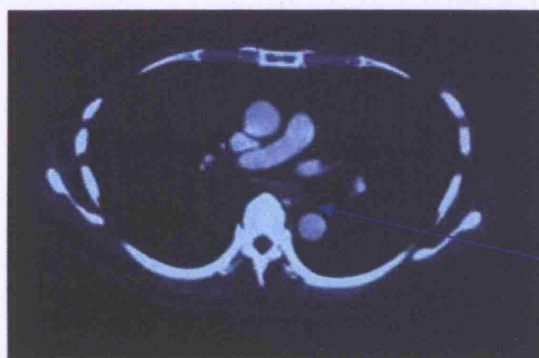
2.4.6 Treatment Responses assessed by CT

CT Responses were seen in 2 patients who had both received cumulated administered activities of greater than 2220 MBq/m² and included a single complete response (unconfirmed) in patient 05 and a single partial response in patient 07. Results for all patients are shown in Table 2.8.

Table 2.8: CT responses according to administered activity

Patient no.	Dose level (MBq/m2)	CT Response according to WHO criteria	Comment
01	370	-	Clinical Progression
02	370	PD	
03	370	SD	
	740	SD	Clinical Progression
04	740	SD	
	1480	SD	
	2220	SD	
	370	SD	
05	740	PR	
	1480	CR	
06	740	SD	
07	1480	SD	
	2220	PR	Clinical improvement
	2960	-	Not available for imaging
08	740	PD	Scanned at referring Hospital
09	740	SD	
	1480	SD	
	2220	PD	

Image 2.1: CT Response of mediastinal lymphadenopathy in patient 05 following 2220 MBq/ m² in two fractions.



Mediastinal
lymphadenopathy

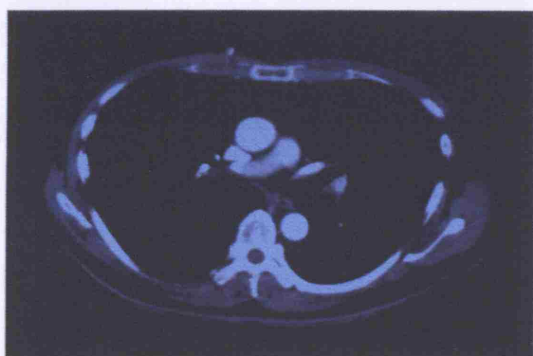


Image 2.2: CT Response in para-spinal lymphadenopathy in patient 05

following 2220 MBq/ m² in two fractions.



Para- spinal lymphadenopathy

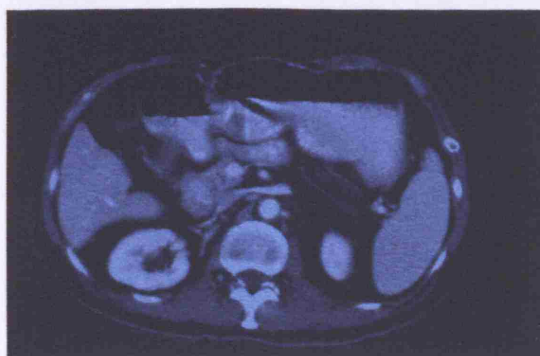
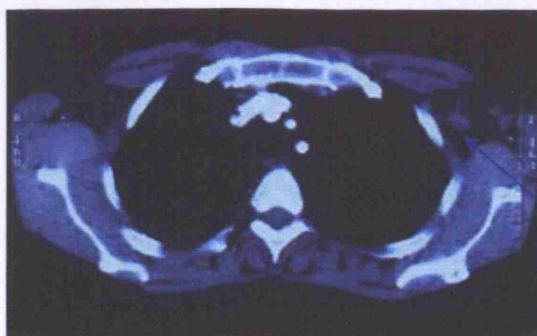
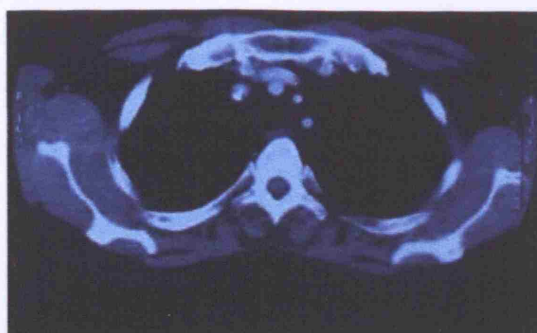


Image 2.3: Good partial response in left axillary lymphadenopathy in patient 07

following 3700 MBq/ m² in two fractions



Left axillary lymphadenopathy



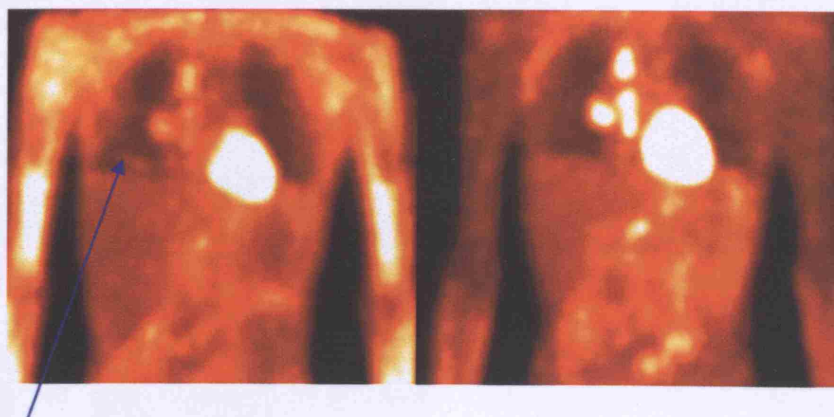
2.4.7 Treatment Responses assessed by FDG-PET

Treatment responses measured by FDG-PET were assessed using both qualitative visual assessment and also using the semi-automated programme outlined above. Results are shown in Table 2.9. By visual assessment, FDG PET responses were seen in 4 patients and a further transient PET response seen in another (patient 09). PET responses were seen at all the administered activity levels. In comparison, the quantitative FDG-analysis revealed some differences. In patient 03 a partial response was reported upon visual assessment, but quantitative analysis suggested progressive disease and the clinical progression seen at this time was supportive of the improved accuracy of the quantitative system. In patient 05 where a partial response was reported following therapy at the 740 and 1480 MBq/ m² dose levels, the quantitative analysis reports a complete response due to erroneous growth into adjacent FDG-avid myocardial tissue, a weakness of the region growing algorithm. Disagreement was also seen in the response of patient 06 with visual assessment suggesting stable disease and quantitative assessment suggesting progressive disease. Re-assessment of the FDG images would appear to support the quantitative assessment and is shown in Image 2.4

Table 2.9: Treatment responses assessed by FDG-PET

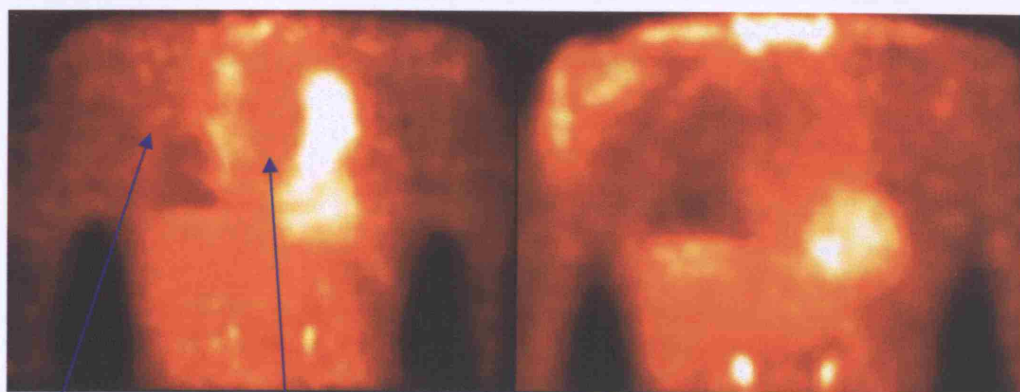
Patient no.	Dose level (MBq/m²)	Visually assessed FDG-PET	Quantitative FDG-PET Counts as percentage of baseline	Quantitative FDG-PET assessment	Comment
03	370	PR	67	PR	
	740	PR	139	PD	Clinical Progression
04	740	PR	67	PR	
	1480	CR	0	CR	
	2220	CR	0	CR	
	370	PR	53	PR	
05	740	PR	0	CR	FDG avid heart uptake
	1480	PR	0	CR	FDG avid heart uptake
06	740	SD	394	PD	
07	1480	-	-	-	
	2220	PR	1.22	PR	Clinical improvement
	2960	-	-	-	Not available for imaging
09	740	SD/PD	137	PD	
	1480	SD	154	PD	
	2220	PD	297	PD	

Image 2.4: Discrepancy between visual FDG-PET analysis and quantitative FDG analysis in patient 06. Baseline image on left appears less FDG-avid than one month response image on right, although the degree of background uptake appears different between the two images.



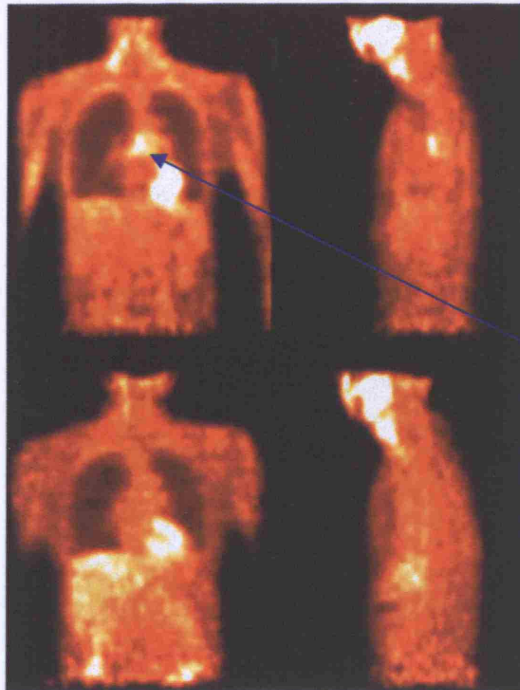
Mediastinal lymphadenopathy

Image 2.5: FDG response in patient 04: Resolution of left sided mediastinal LN and also right upper mediastinal LN following 4440 MBq/m² in 3 fractions.



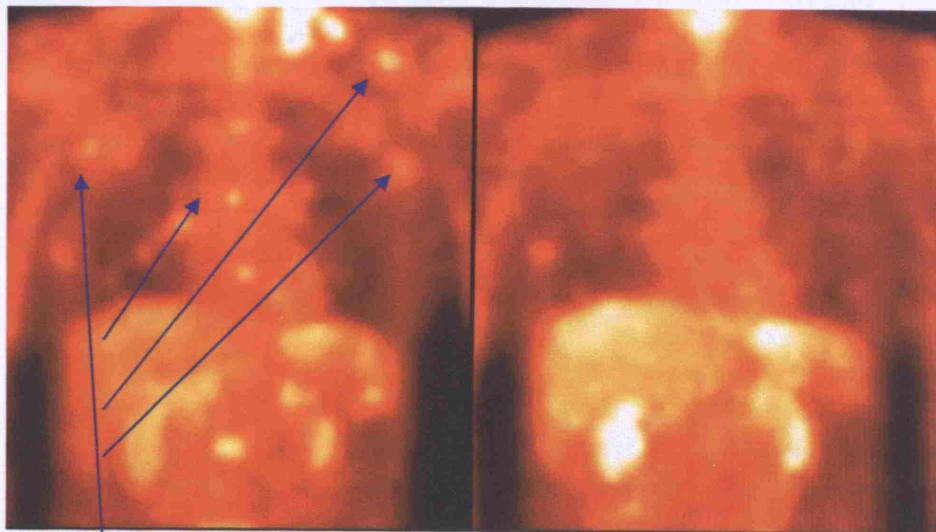
Right and left sided mediastinal lymphadenopathy

Image 2.6: FDG Response in patient 05: Resolution of mediastinal LN following 2220 MBq/m² in two fractions



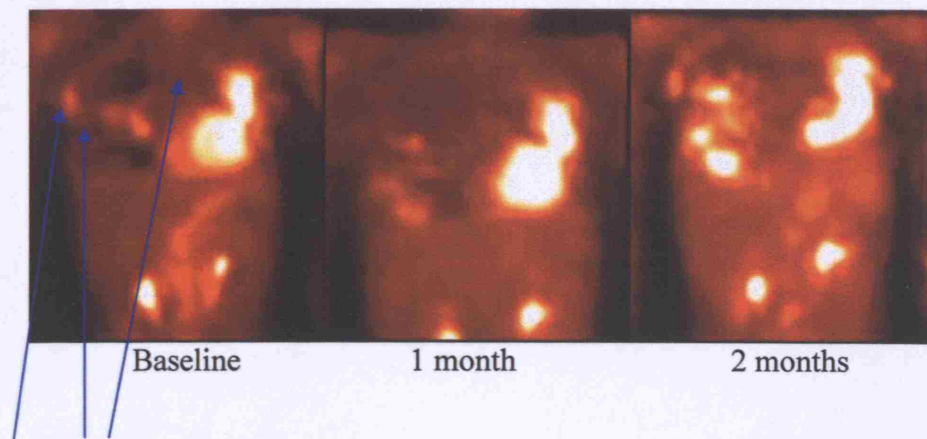
Mediastinal lymphadenopathy

Image 2.7: Partial PET response in patient 07: Reduced FDG avidity in multiple nodal areas including left SCF, both axillae, mediastinum and right rib.
Administered activity 3700 MBq /m² in 2 fractions.



Axillary, supraclavicular and mediastinal lymphadenopathy and right rib metastasis

Image 2.8: Partial PET response in patient 09 following 4440 MBq/m² in three fractions. Early relapse was seen at 2 months however and the patient was removed from the study for disease progression. On quantitative analysis there was a reduction in FDG- avid counts at 1 month, though not to less than baseline, followed by a further rise suggesting continued progressive disease at both time points over baseline.

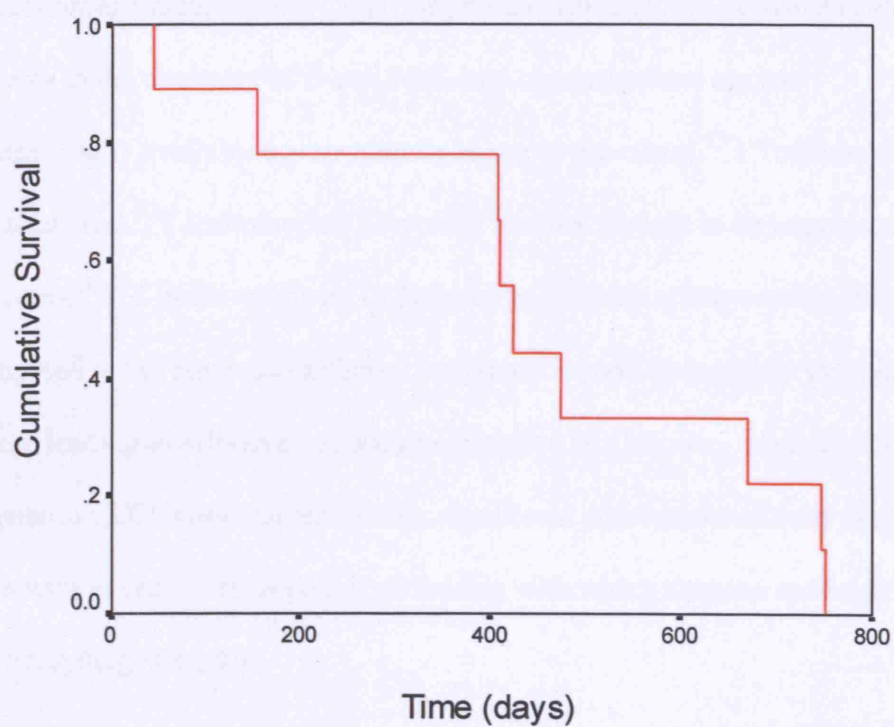


Right axillary lymphadenopathy and
bilateral parenchymal lung disease

2.4.8 Overall Survival

Survival times were calculated from the start of treatment and at the time of writing all the patients treated had died. The median survival of the patients was 424 days (95% confidence interval 377-471 days). As survival was not an endpoint in this study, no major conclusions can be drawn from this survival data.

Figure 2.4: Kaplan-Meier survival of patients on CHT-25 study



2.5 Discussion

Radioimmunotherapy using CD20 targeting antibodies has shown considerable success in the treatment of B-cell NHL and currently there are two commercially available agents already in use in the clinic, ^{131}I Tositumomab (*Bexaar*) and ^{90}Y Ibritumomab (*Zevalin*). Factors thought to be important for the success of RIT in the treatment of lymphoma are their relative radiosensitivity compared to the common epithelial malignancies and their highly vascular nature leading to effective antibody penetration into tumour. Additionally with respect to CD20 targeting antibodies, significant anti-tumour activity is already seen with naked CD20 targeting antibodies with which targeted radiation may have a synergistic effect.

The interleukin 2 receptor alpha chain is an attractive target for RIT due to its limited physiological expression combined with its abnormal expression in a variety of lymphomas and Hodgkin's disease. In this phase I/II dose escalation study we have used a chimeric monoclonal antibody to the α chain of the IL-2R labelled with ^{131}I to treat patients with refractory IL-2R expressing lymphomas.

Acute toxicity following therapy has been limited with low grade infusion related toxicities seen in only a small number of patients. Delayed hypothyroidism has been seen in a single patient undergoing repeated therapies, presumably as a result of non-specific ^{131}I uptake by the thyroid, despite thyroid blockade using potassium iodide (see activity biodistributions in Chapter 3). In common with other studies of RIT, the prime toxicity observed has been delayed haematological toxicity occurring 4-6 weeks after antibody administration.

Considering each therapy individually, the observed haematological toxicity has been dose dependent and the relationship between the two investigated using regression analysis. A significant association between administered activity and observed toxicity has been found for platelets, total white cell count and neutrophils. No significant associations were found for haemoglobin and lymphocytes and this probably reflects the lack of red cell toxicity, and in addition the universal lymphopenia seen at all the dose levels used in this study. In general, significant marrow toxicity has only become evident above the 740 MBq/m² dose level suggesting that the non-myeloablative MTD lies above this level. Marrow reconstitution has only been required in a single patient who received 2960 MBq/m² on a background of repeated therapies with a total administered activity of 6.4 GBq. This patient failed to engraft and subsequently died during a prolonged period of neutropenia which was complicated by *Pneumocystis carinii* pneumonia. Following this toxic death, this highest dose level was removed from the protocol and all subsequent patients commenced therapy at 740 MBq/m².

In order to assess the effects of cumulative marrow radiation exposure resulting from repeated therapy, the observed haematological toxicity has also been correlated with cumulated administered activity using regression analysis. Significant correlations were found for platelets, total white cell count and neutrophils, but not for haemoglobin and lymphocytes, as before. The correlation between cumulative administered activity and haematological toxicity did not appear any stronger than that seen with individual administered activity. One possible explanation for this is that a reasonable degree of

repopulation of marrow stem cells may be occurring between treatments, which was at least 1 month in all patients.

Given the heavily pre-treated nature of the study population it is encouraging that evidence of treatment responses have been seen at this early stage. FDG-PET responses have been seen in four patients and CT responses in two. FDG-PET responses have been seen at all dose levels including the lowest dose level of 370 MBq/m². The response seen at 370 MBq/m² is surprising, as tumour can only have received a modest dose of radiation. Although we have proposed that tumour kill with ¹³¹I-CHT-25 is due to selective radiation delivery alone, since the amounts of antibody used are so small in comparison to those used in immunotherapy, it is tempting to speculate whether an antibody-dependent mechanism may also be in part responsible. Although blockade of IL-2R α using naked murine anti-tac has proved to be an effective mechanism of tumour control in HTLV-1 associated adult T-cell leukaemia/ lymphoma (383), in this tumour type IL-2R auto-stimulation is an integral part of their pathophysiology resulting from HTLV-1 integration into the genome (371). However, during normal physiology, activation of the IL-2R by IL-2 is an important growth signal to T-cells and also acts as an anti-apoptotic signal (292). This raises the question as to whether aberrant expression on tumour cells may be contributing to their growth. In addition it has been suggested that IL-2R α targeting antibodies may lead to cell death by mechanisms other than cytokine depletion. In a murine model of adult T-cell leukaemia, ligation of the IL-2R α by anti-tac antibody has been proposed to lead to cell death by mechanisms including ADCC and the direct induction of apoptosis (384). It is presently unknown

whether blocking this growth signal is enough on its own to lead to clinical responses in IL-2R expressing lymphomas as there is no published clinical data using naked CHT-25 or other IL-2R α antibodies to treat these patients. In addition, even if IL-2R α blockade was not adequate to lead to cell death on its own, there may be synergy between loss of IL-2R mediated cell signalling and radiation, which might enhance the efficacy of RIT using ^{131}I -CHT-25.

In the assessment of treatment responses using FDG-PET, a number of discrepancies have been observed between the assessment of response using qualitative visual assessment and that by semi-quantitative image analysis. In some instances this may be a reflection of the limitations of the image analysis software. In particular a documented difficulty using this software is where FDG-avid tumour lies in close proximity to FDG-avid normal tissue (patient 05). In other circumstances however, this software appears to offer a truer reflection of the physiological response to tumour where clinical progression has supported evidence of progressive disease on FDG-PET when assessed quantitatively rather than the stable disease reported by visual assessment (Patient 03). CT responses have only been seen at the higher dose levels with the two documented responses seen at cumulated activities of greater than or equal to 2220 MBq/m².

2.6 Conclusions

^{131}I labelled CHT-25 has been administered to nine patients with refractory/relapsed lymphoma in the dose-escalation phase of this phase I/II study. The toxicity profile in this group of patients has largely comprised of delayed blood

cytopenias which is in keeping with other studies of RIT. Although a single toxic death has been seen, the MTD has not yet been established. Since the responses seen in the patients treated so far have been achieved without the need for marrow reconstitution, this early data suggests that ^{131}I -CHT-25 may show clinically useful efficacy in this group of patients at dose levels which do not require stem cell support.

3 Radiation Dosimetry and Biodistribution studies of Chimeric

CHT-25 and Murine A5B7 antibodies: Correlation with Observed

Haematological Toxicity

3.1 Introduction

Many years of external beam radiotherapy (EBRT) indicate that a clear relationship exists between the radiation absorbed dose and early and late normal tissue toxicity and tumour response, even for specific tumour types (85;86). Maximal tumour doses that also allow for acceptable levels of normal tissue toxicity are achieved by patient-specific calculations of radiation absorbed dose and the intended radiation doses determined by response-morbidity relationships observed in studies of earlier patients.

By contrast, chemotherapy is normally delivered using a single dose with minor modifications depending upon the mass or body surface area of patients. During their development doses are usually determined by dose-escalation studies which involve administering therapy according to patient body surface area or weight and determining the maximum tolerated dose (MTD). Similarly, in RIT, dose escalation studies are commonly used to determine a safe administered activity for future use. However dose escalation studies, although easy and safe to implement, do not *directly* account for physiological variability in the metabolism of drugs from one patient to the next. Therefore they may not be the most effective way to define treatment protocols, especially where there is significant heterogeneity in drug biodistribution between patients.

In RIT, emitted gamma radiation from therapeutic radionuclides allow for the biodistribution of radioactivity to be determined in individual patients much more easily than for systemic chemotherapy. As a result, in phase I/II studies, detailed biodistribution information is often available. Such information is commonly used to compare normal tissue and tumour distributions of different antibody-radionuclide combinations and allows for a *direct* estimate of the variability in activity pharmacokinetics between patients. Moreover, since the absorbed dose in normal tissue and tumour is also determined by the biodistribution of radioactivity using this information, it is also possible to make estimates of absorbed dose in normal tissues and tumour following RIT

One approach to radiation dosimetry in RIT is to perform this retrospectively allowing for the construction of dose-response curves which can be used for defining future treatment protocols. However the ability of such dose response curves to predict future absorbed doses in individual patients is only likely to be reliable when there is relatively little variation in pharmacokinetics between patients. Furthermore, the most commonly used method to determine absorbed dose, the MIRD schema, only allows for an estimate of absorbed dose for a *population* of patients. This is because MIRD makes a number of significant assumptions including homogeneous antibody biodistribution in normal tissues and that all patients have a standard geometry (148).

Another approach, which may be more appropriate where biodistribution studies demonstrate significant inter-patient variability, is customised patient dosing

using individual patient biodistribution information which has been determined previously. High inter-patient variability in urinary and whole body clearance has commonly been seen with ^{131}I labelled antibodies (161;162). As a result ^{131}I Tositumomab (*Bexaar*) has been developed so that patient-specific activities are administered depending upon the whole body clearance times of tracer doses of radiolabelled antibody in order to deliver a fixed whole body dose (385). In this case the use of whole body absorbed dose as a surrogate for marrow absorbed dose is reasonable since the whole body absorbed dose from ^{131}I radiolabelled antibodies is known to be responsible for a significant proportion of the total marrow absorbed dose (92;93). This association between whole body clearance and marrow clearance has recently been verified by Boucek et al (386).

In this chapter I have investigated the clinical biodistribution and dosimetry of ^{131}I -CHT-25 and ^{131}I -A5B7. Data for ^{131}I -A5B7 was collected during a phase I/II clinical study treating patients with relapsed/ refractory CEA expressing tumours. In this study antibody was given in combination with the antivascular drug Combretastatin-A4-30 phosphate (CA4-P). Data for ^{131}I -CHT-25 has been collected during the phase I/II dose escalation study described in Chapter 2. Both antibodies are currently administered on the basis of body surface area and the MTD for ^{131}I -A5B7 monotherapy has previously been defined in a dose escalation study (232). The biodistribution of both antibodies has been quantified in three ways. Normal organ and tumour biodistributions have been measured using serial SPECT imaging, blood biodistribution has been measured by gamma counter measurements of serial blood samples and finally whole body clearance has been measured using external dose rate measurements.

Chimerisation is reported to increase the circulating half-life of whole antibodies in comparison to murine ones and this has important implications for radiation dosimetry. One concern when utilising such antibodies is that this will markedly increase the radiation exposure of normal tissues, in particular bone marrow, which may lead to unacceptable toxicity. By comparing the murine antibody A5B7 with the chimeric antibody CHT-25 I have investigated the effects of chimerisation upon biodistribution and absorbed dose, using the MIRD schema, in both normal tissue and tumour. Finally I have also presented data upon the relative immunogenicity of ^{131}I -CHT-25 in comparison to ^{131}I -A5B7.

Given the wide inter-patient variability reported in biodistribution of ^{131}I labelled antibodies, I have also examined whether retrospective radiation dosimetry and the definition of a single dose is the most appropriate way to develop these antibodies for future use, or whether individual patient dosing using tracer doses might be more appropriate. In order to do this I have compared the ability of simple but individual patient biodistribution data with MIRD absorbed doses to predict haematological toxicity.

3.2 Materials and Methods

3.2.1 Quantitation of Radioactivity

The methods used to quantify radioactivity in normal tissue and in tumour were identical for both ^{131}I -CHT-25 and ^{131}I -A5B7, although the shorter biodistribution times of A5B7 meant that the period of observation following therapy was often shorter than for CHT-25. In addition, no urinary activity measurements were made for A5B7

¹³¹I Whole body pharmacokinetics

Sequential whole body emitted dose rate measurements were taken daily by a member of the Royal Free Hospital radiation protection department at a fixed geometry from all patients using a hand held dosimeter.

¹³¹I activity in blood and urine

2.5 mls of blood were taken into an EDTA blood tube to determine ¹³¹I activity at a number of time points following the completion of the antibody infusion. The number of samples taken were fixed for A5B7 but for CHT-25, where administered activity varied between patients, the number depended upon the administered activity, with more observations made following higher activities. For CHT-25 patients an additional daily 24-hour urine collection was performed following infusion of antibody and the total volume recorded. A 2 ml aliquot was subsequently collected in a plain container and the rest discarded. All samples were collected by research nurses and remained in the patients' room in a lead-lined box until the patients' discharge. They were then taken and stored in an approved radioactive store in the Department of Medical Physics (supervised by a representative of Radiation Protection) to decay until at safe levels for analysis. ¹³¹I levels in blood and urine were then measured retrospectively using a LKB Wizard (Pharmacia) gamma counter in the department of oncology, decay corrected and results expressed as a percentage of the injected activity/kg. S.Parker performed counting of samples under the guidance of Dr A.Green.

¹³¹I activity in visceral organs and tumour

The activity in tumour and visceral organs (liver, lung, heart, spleen and kidney) was determined from gamma camera imaging. Patients were imaged using an ADAC Vertex Gamma Camera and images taken to quantify the distribution of ¹³¹I. Single Photon Emission Computed Tomography (SPECT) images were acquired at various time points post administration of antibody. For CHT-25 patients scans were performed at 4 hours post administration of antibody and then daily until day 4; further daily imaging was additionally performed up to day 15 depending upon administered activity. For A5B7, SPECT imaging was performed at 4 hours, 24 hours, 48 hours and 72 hours. Optional scanning at 96 hours was performed depending upon remaining levels of radioactivity. SPECT scans were then reconstructed and corrected for Compton scatter and attenuation. S.Parker performed Scanning and reconstruction of SPECT data under the guidance of Dr A. Green.

The method used to estimate ¹³¹I activity in tumour and normal tissues involved drawing regions of interest, of at least 9 voxels, placed in triplicate in areas of tumour and normal tissue (lung, liver, heart, kidney and spleen) resulting in the mean counts per voxel for each region as described by Green et al (144). A calibration factor, obtained from scanning a phantom containing known amounts of ¹³¹I under clinical conditions was then used to convert counts per voxel into radioactivity per kg. This value was decay corrected and divided by administered activity to calculate the percentage injected activity/kg in tumour and normal tissues (see Appendix 1 for example spreadsheet and detailed outline

of calculations). My input to this process was in defining the regions of interest and subsequent data analysis.

3.2.2 Pharmacokinetic Calculations

Decay characteristics for whole body and visceral organs were modelled mono-exponentially, whereas blood activity was modelled bi-exponentially and was performed using the Excel solver function. In such a way the decay characteristics for each patient, and for each group of patients as a whole, were calculated. Remainder of the body decay characteristics were calculated by removing activity in single organs from the whole body activity at each time point and then modelled mono-exponentially in a similar fashion.

3.2.3 Radiation Dosimetry

An estimate of normal tissue absorbed doses has been performed for the study populations using the observed biodistribution characteristics and the MIRD schema. The critical value to enable this to be performed is the generation of normal organ and tumour residence times, as outlined in Chapter 1.

Determination of Normal Organ Residence Times

The residence times in whole body, visceral organs and blood were estimated by integration of the population pooled exponential decay characteristics determined above. For visceral organs and blood, where measured values represent activity/litre, data was converted to the number of radioactive disintegrations per kg depending upon the specific density of the organ (assumed to be 1 for all organs except lung which was assumed to have a

specific density 0.2). In turn, whole organ residence times (MBq.hrs/ MBq) have been calculated using standard organ masses. Marrow residence time was assumed to represent 0.36 that of whole blood (145). The determination of residence times for tumour in which activity distributions did not fall exponentially with time were made using the trapezoidal method. In this case, in order to estimate the remaining AUC for time points beyond the observation period, mono-exponential decay was assumed to have occurred at a rate equating to the terminal blood clearance in each patient. The observed and estimated residence times were then summed to give the total tumour residence time.

Radiation Dosimetry Calculations using MIRD

Normal tissue residence times were entered into Olinda software in order to determine the total absorbed doses and dose rates in normal tissue (387). For estimates of tumour absorbed dose the sphere model in Olinda was used to determine the absorbed self dose. In view of the variable location of tumours in relation to normal body organs this facility cannot assess the contribution to tumour absorbed dose from whole body radiation, and in addition the model assumes that all tumours are spherical in shape.

3.2.4 Immunogenicity

In order to determine the immunogenicity of the antibodies, 5 mL of blood in a plain clotted tube were taken for measurement of HAMA levels by ELISA pre-study and then once weekly for 8 weeks after administration of antibody. These values were compared to known positive controls for A5B7, although no

positive controls are currently available for CHT-25. All ELISA studies were performed by Dr S. Sharma and her team.

3.3 Results I: Normal Organ Biodistributions and Dosimetry

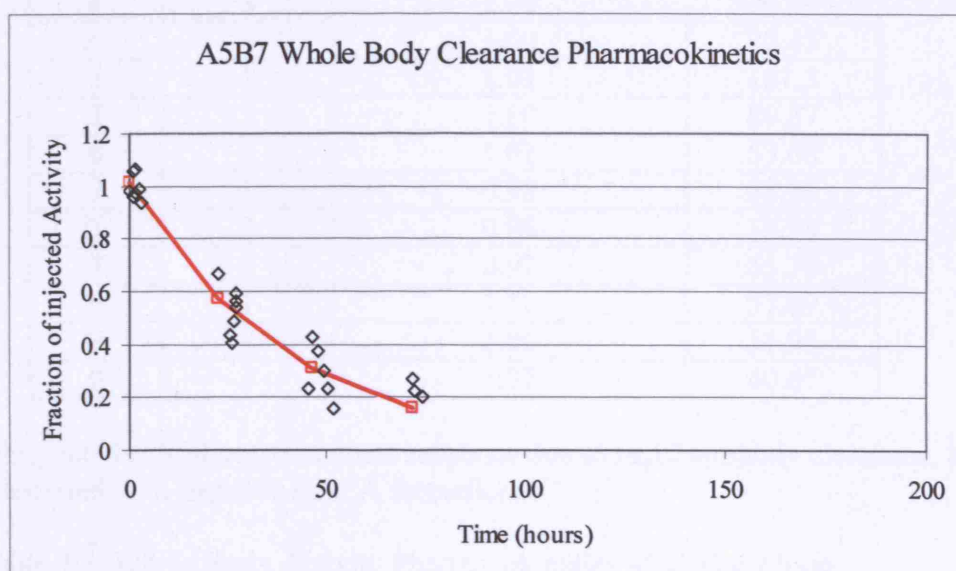
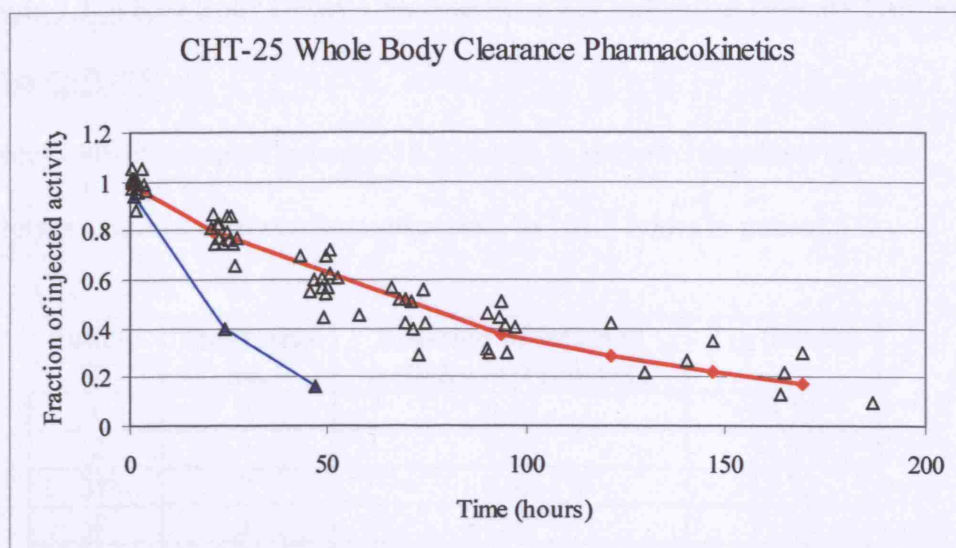
Data was collected following the treatment of 9 patients using CHT-25 with a total of 18 antibody administrations, and from 6 patients using A5B7 with a total of 7 antibody administrations.

3.3.1 Whole Body Activity Biodistributions

Whole body emitted radiation dose rates for ^{131}I CHT-25 and ^{131}I A5B7 are shown in Figure 3.1 and revealed a markedly higher half-life for CHT-25 compared to A5B7. Mono-exponential modelling of the pooled data for each patient population revealed decay half-lives of 66.9 hours and 26.9 hours respectively.

Figure 3.1: Whole Body Activity Levels For Individual A5B7 and CHT-25 Therapies Assessed Using Hand Held Dosimeters And Fixed Geometry.

Modelled decay characteristics are shown in red. Blue data points in the CHT-25 graph are from a patient in whom an anti-idiotypic immune response was suspected leading to rapid whole body activity clearance. This data was excluded from the pharmacokinetic analysis.



Mono-exponential modelling of whole body activity pharmacokinetics for individual patients has been performed and is shown in Tables 3.1 and 3.2 for CHT-25 and A5B7 respectively.

Table 3.1. Whole Body Decay Characteristics For Individual Patients Treated With CHT-25.

Decay half-lives varied between 18.22 hours in patient 3 in whom an anti-idiotypic immune response was suspected, to 101.3 hours in patient 5.

Patient ID	Treatment no.	Fraction of injected Activity at Time zero	T_{1/2} (hours)
1	1	0.89	89.13
2	1	1.03	56.54
3*	2	0.98	18.22
4	1	1.00	79.95
4	2	0.99	74.33
4	3	1.00	96.69
4	4	1.00	72.45
5	1	1.01	101.3
5	2	1.00	80.87
6	1	1.02	53.68
7	1	0.98	58.56
7	2	0.98	75.58
7	3	0.97	55.79
9	1	1.02	57.69
9	2	1.06	51.98
9	3	1.02	40.67

*Suspected anti-idiotypic immune response due to rapid antibody clearance, but associated with negative HACA formation

Table 3.2: Whole Body Activity Pharmacokinetics Modelled Mono-Exponentially For A5B7.

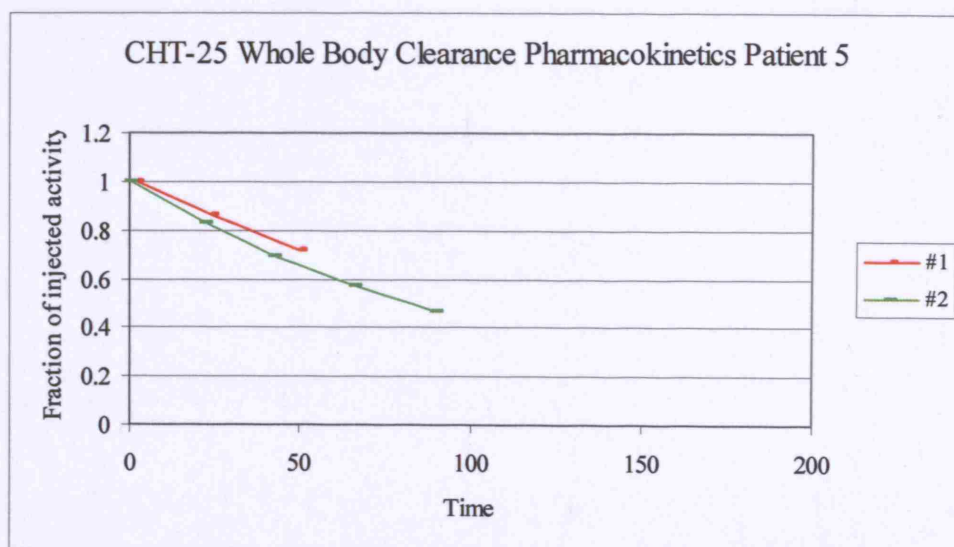
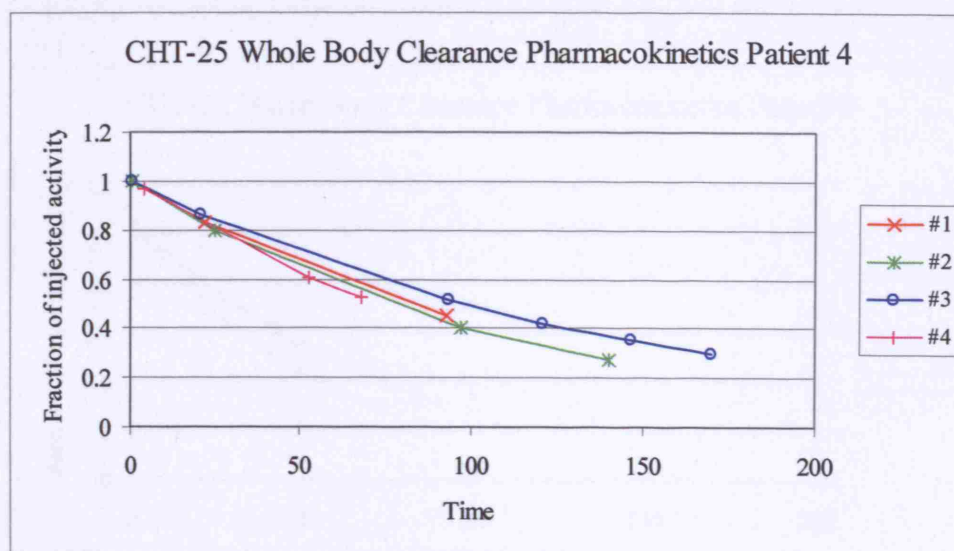
Decay half-lives varied from 18.87 hours in patient 4 to 38.91 in patient 6.

Patient ID	Treatment no.	Fraction of injected activity at T=0	T_{1/2} (hours)
1	1	1.09	26.42
2	1	1.04	32.96
3	1	0.99	21.65
4	1	1.04	18.87
4	2	1.12	22.17
5	1	0.99	32.66
6	1	0.99	38.91

Whole body clearance pharmacokinetics in those patients undergoing repeated therapies with either A5B7 or CHT-25 are shown in Figures 3.2 and 3.3.

Figure 3.2: Whole Body Pharmacokinetics In Patients Undergoing Repeated Therapies With ^{131}I CHT-25.

The highest variability in the half-life of activity decay was 24.24 hours in Patient 4. In this patient, who underwent four treatments, the whole body half-life varied from 96.69 hours to 72.45 hours.



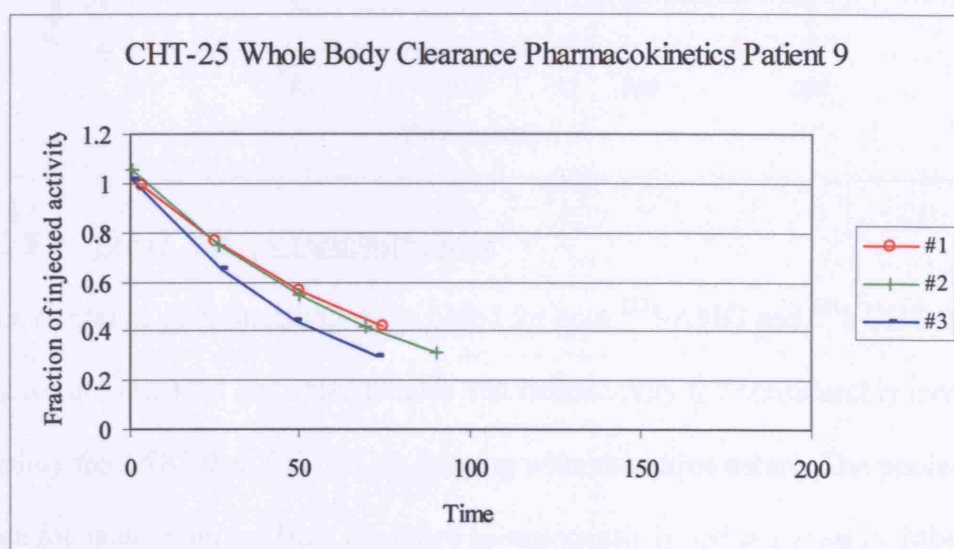
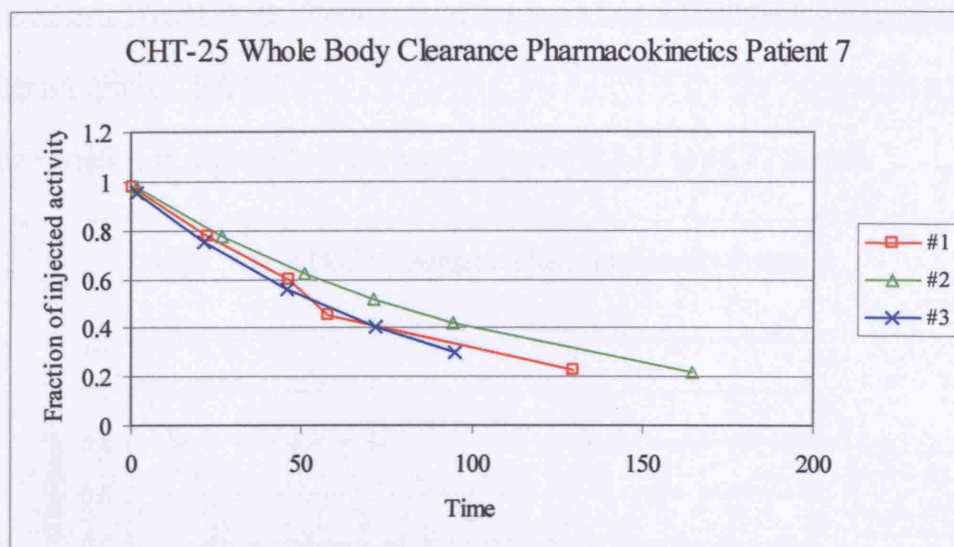
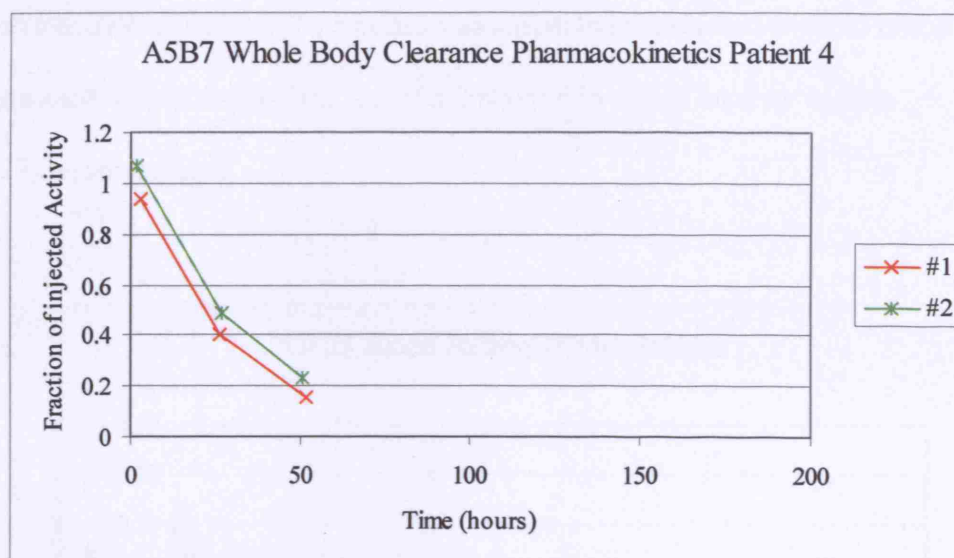


Figure 3.3: Whole Body Pharmacokinetics In Patient 4 Undergoing Repeated Therapy With ^{131}I -A5B7.

The variation in activity half-life was 3.3 hours (22.17 to 18.87 hours).



3.3.2 Blood Activity Biodistributions

The clearance of radioactivity from blood for both ^{131}I -A5B7 and ^{131}I -CHT-25 is shown in Figure 3.4 and demonstrates that radioactivity fell considerably more rapidly for A5B7 than CHT-25, in keeping with its murine nature. The pooled data for each group has been modelled bi-exponentially and is shown in Table 3.3.

Figure 3.4: Blood Activity Pharmacokinetics of ^{131}I -CHT-25 And ^{131}I -A5B7

Revealing More Rapid Decay For A5B7 Than CHT-25.

Blue data points in the CHT-25 graph are from Patient 3 in whom an unconfirmed anti-idiotypic response was suspected which lead to rapid blood clearance. Green data points are from Patient 6 in whom an error in data calibration occurred.

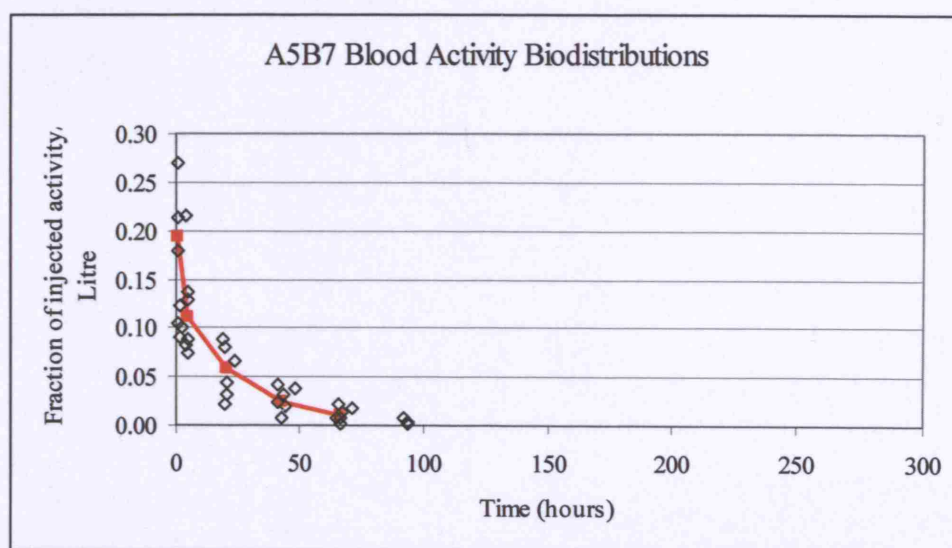
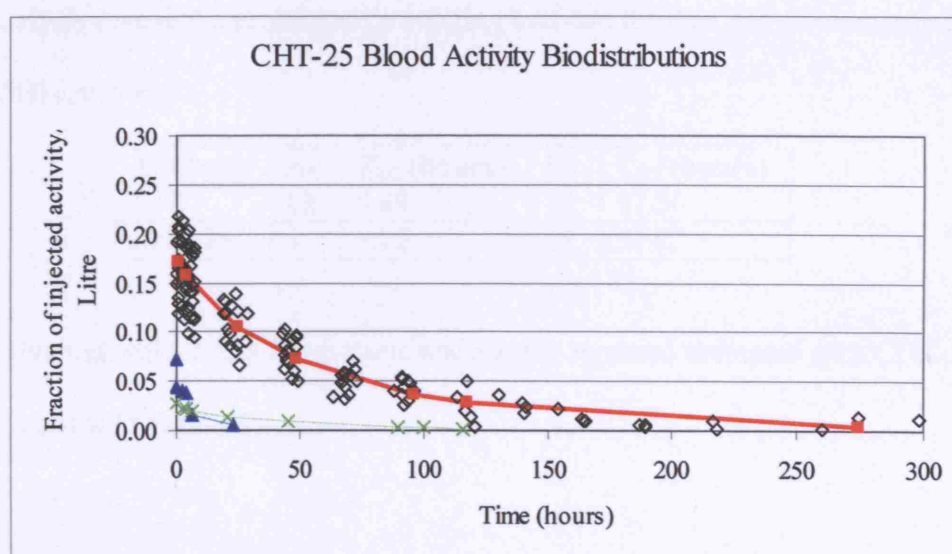


Table 3.3: Blood Activity Pharmacokinetics Determined By Bi-Exponential

Fitting Of Pooled Data Sets For A5B7 And CHT-25 Patients.

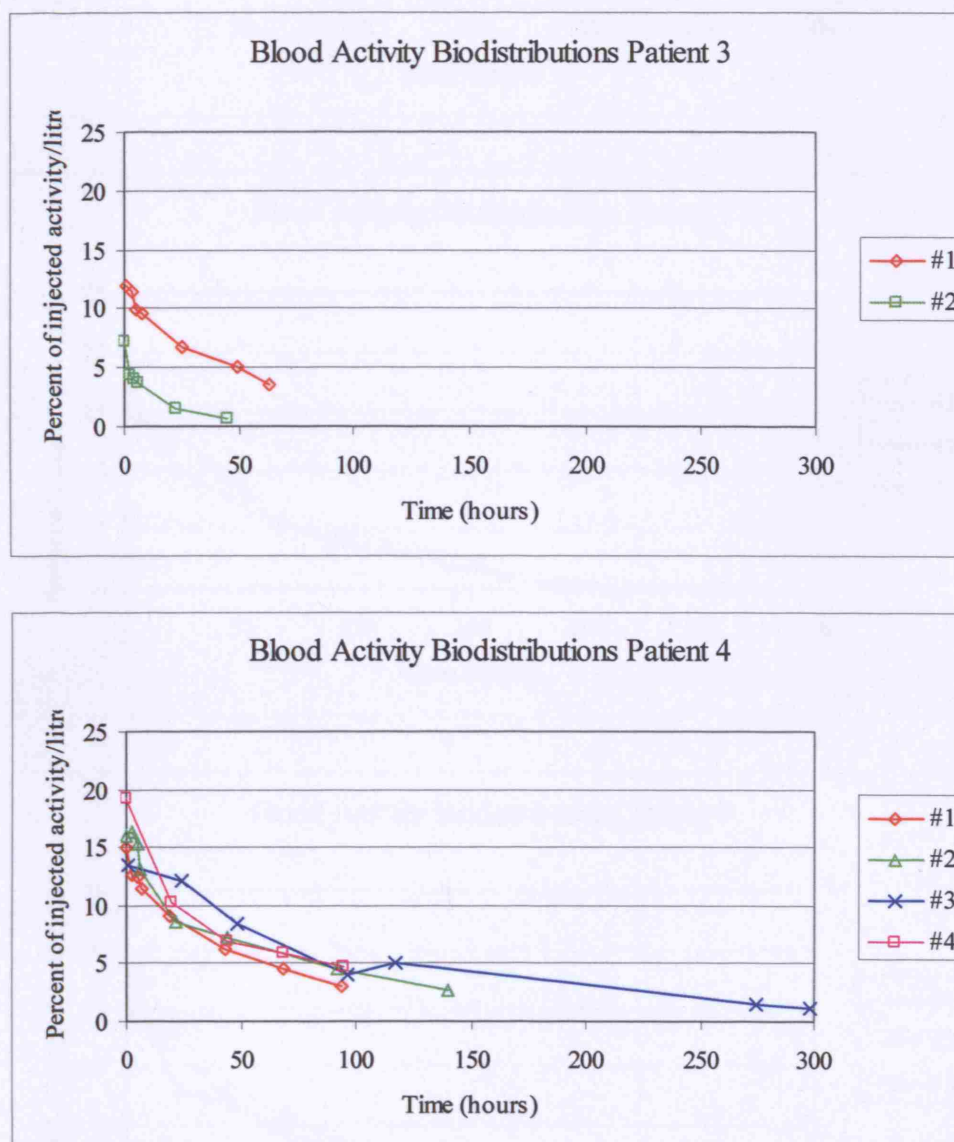
Data from CHT-25 Patient 3#2 was not included due to altered antibody pharmacokinetics secondary to a suspected but unconfirmed anti-idiotypic antibody formation. Data from CHT-25 Patient 6 was also removed due to a suspected error in counting, leading to isolated abnormally suppressed values. Analysis revealed a prolonged circulating half-life for the CHT-25 compared to A5B7 antibody.

Antibody	A₀	T_{1/2}(hours)	B₀	T_{1/2}(hours)
A5B7	13	0.48	13	17.56
CHT-25	4	9.22	14	51.02

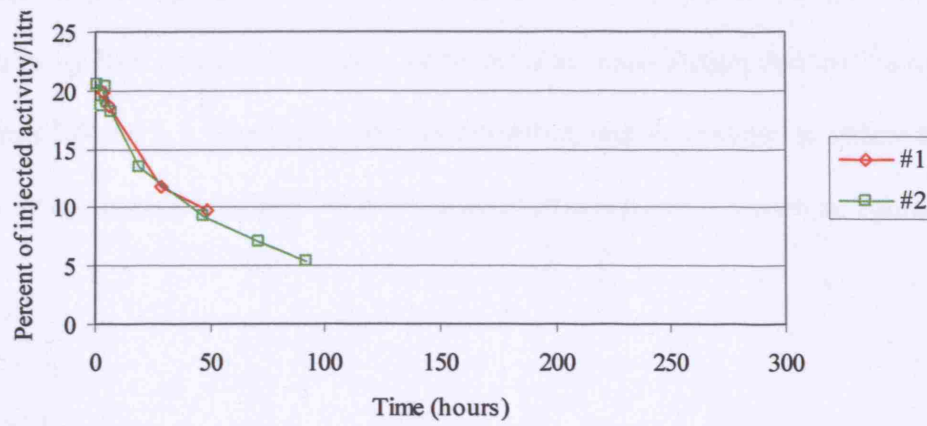
Individual patient data for patients undergoing repeated therapies with CHT-25 are shown in Figure 3.5.

Figure 3.5: Individual Blood Activity Biodistributions In Patients Undergoing Repeated Therapies.

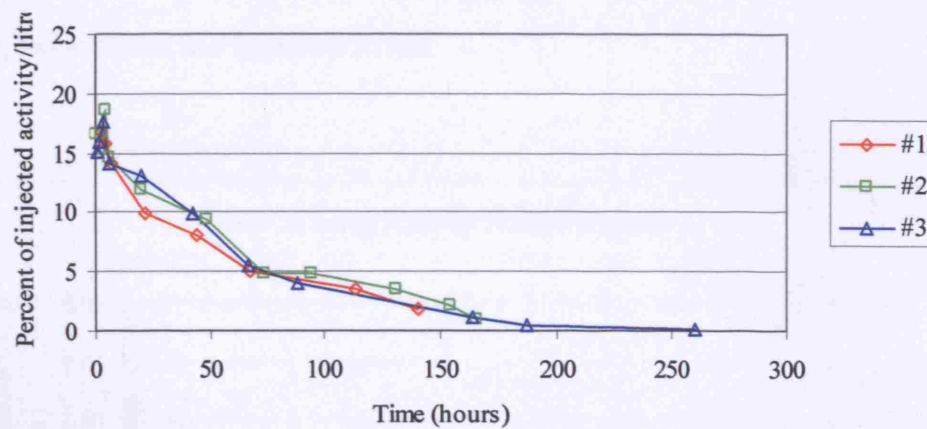
The alteration in pharmacokinetic behaviour though to be the result of anti-idiotypic neutralising antibody formation in Patient 3 is clearly seen. In other patients relatively consistent decay patterns were noted upon repeated exposure.



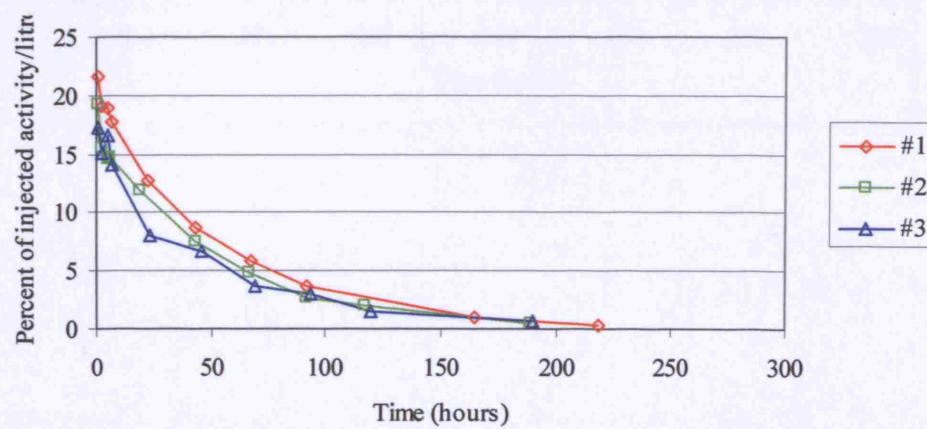
Blood Activity Biodistributions Patient 5



Blood Activity Biodistributions Patient 7



Blood Activity Biodistributions Patient 9

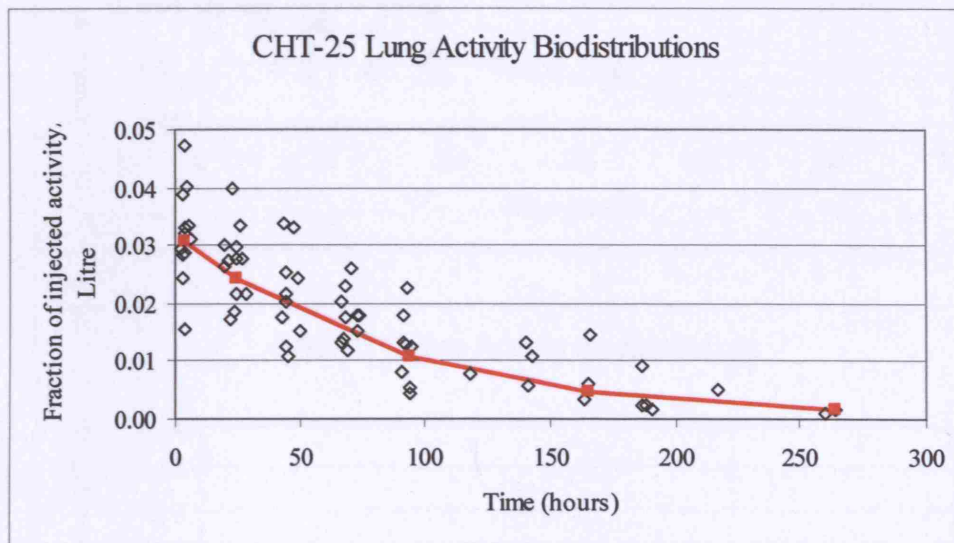


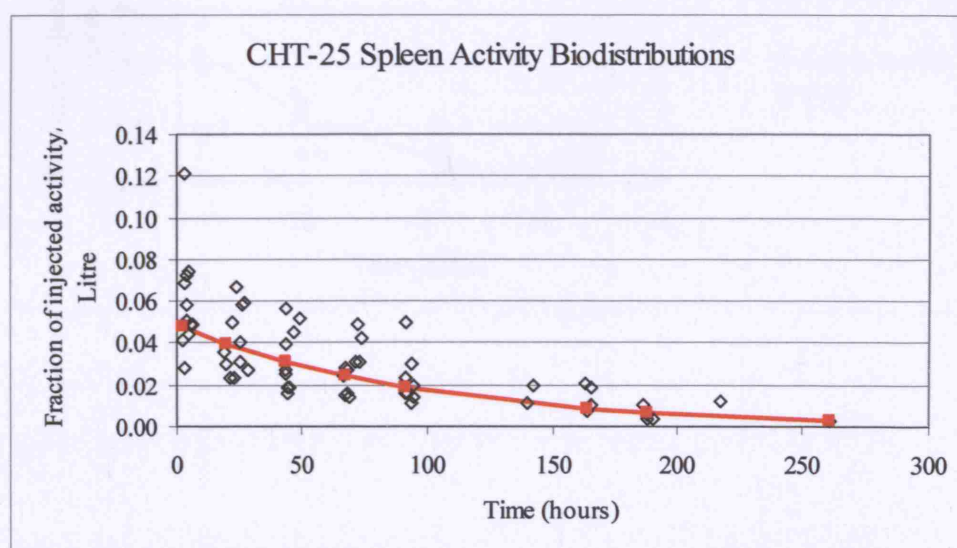
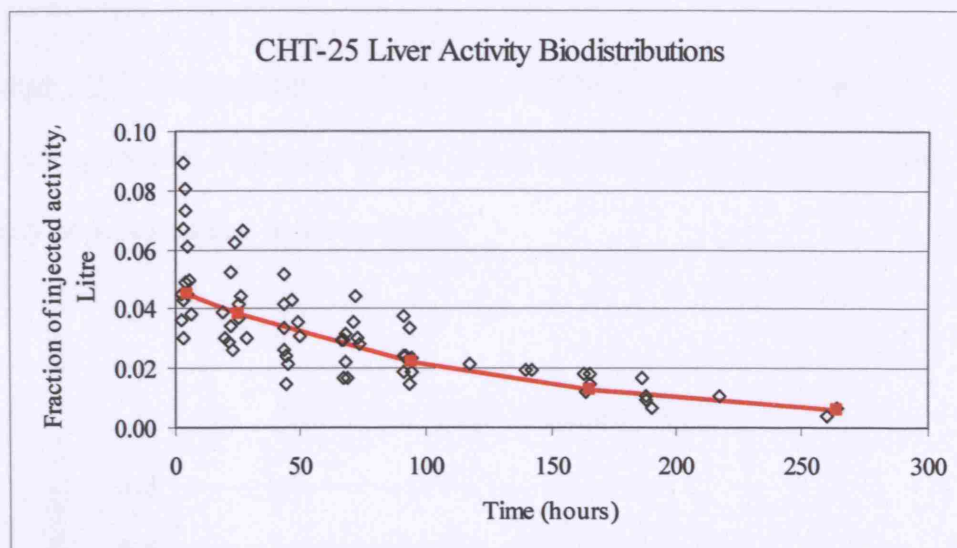
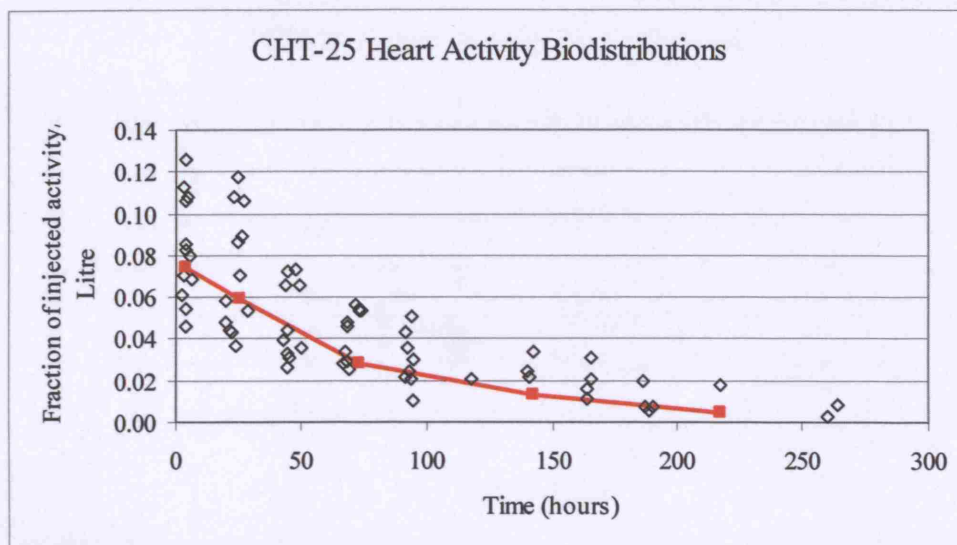
3.3.3 Visceral Organ Activity Biodistributions

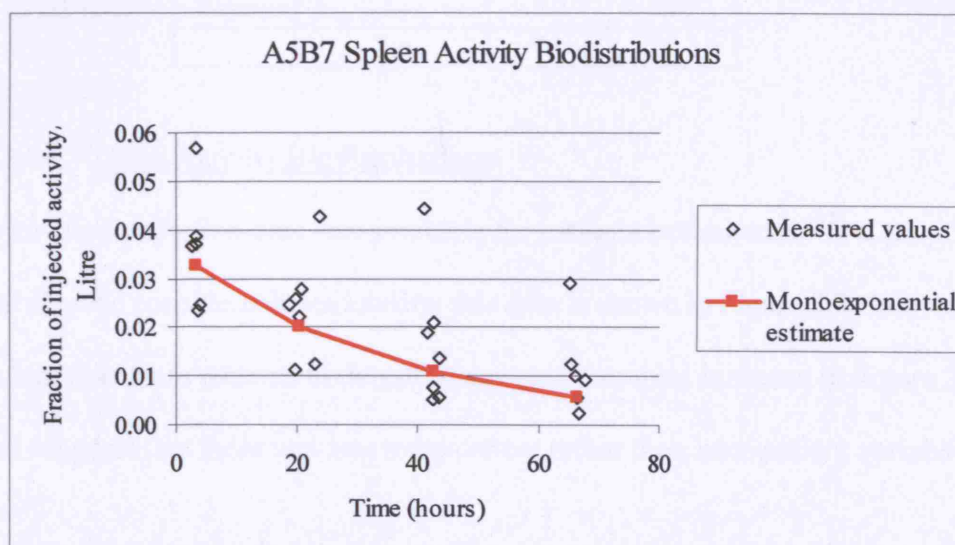
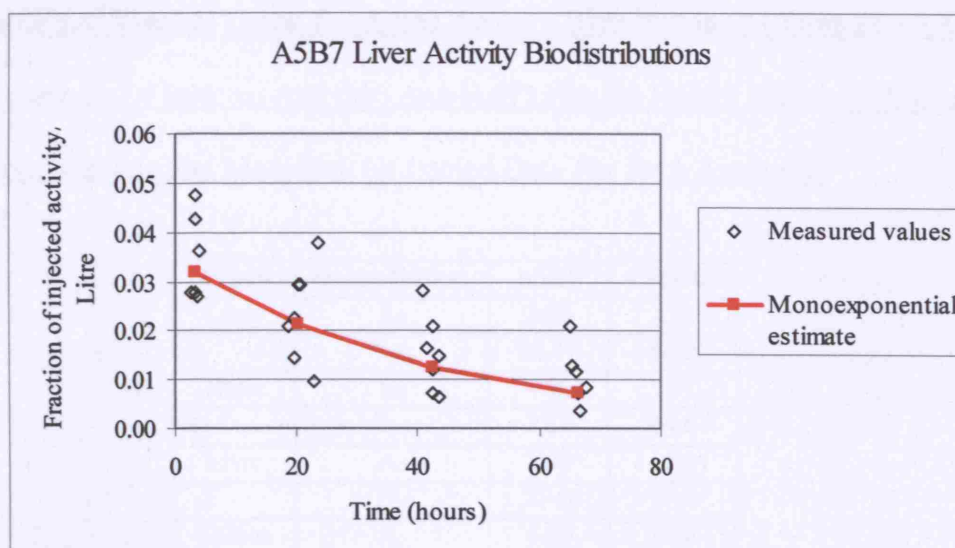
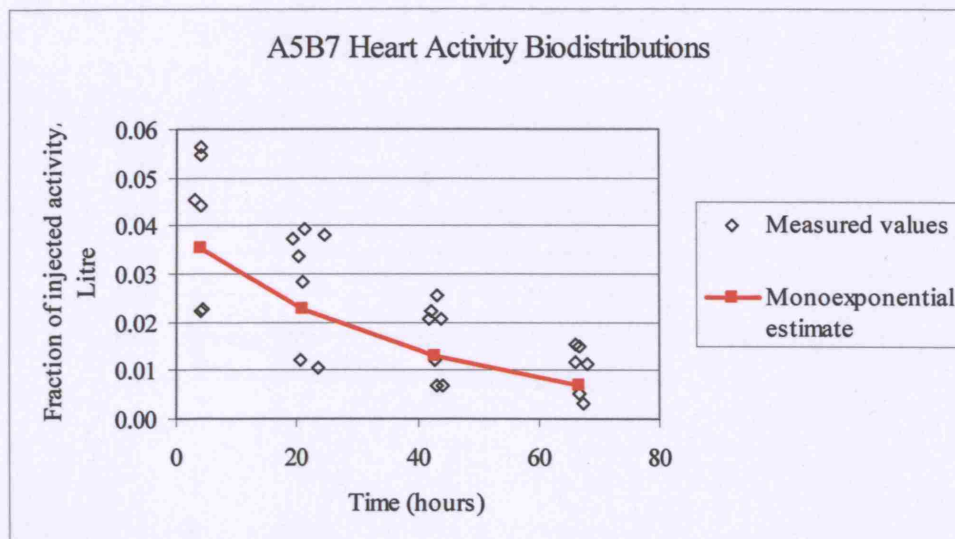
Visceral organ pharmacokinetics were obtained for lung, heart, liver, spleen and kidneys by ROI analysis of serial scatter and attenuated images and are shown in Figures 3.6 and 3.7. Mono-exponential modelling was undertaken to define the activity decay characteristic for each study population and is shown in Table 3.4.

Figure 3.6: Visceral Organ Antibody Pharmacokinetics for CHT-25 patients.

Mono-exponential fitting was used to determine decay characteristics for the study populations and is shown in red.







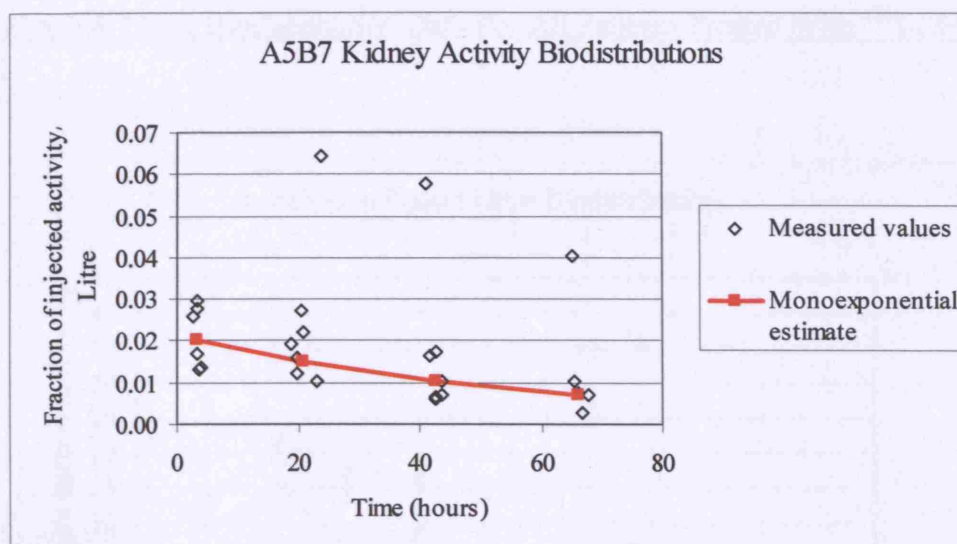


Table 3.4: Visceral Organ Estimated Activity Distributions At Time Zero (As Percentage Of Injected Activity) And Half Lives (In Hours) Estimated Using Mono-Exponential Modelling Of Pooled Data For Each Antibody.

Organ	Decay Indices	A5B7	CHT-25
Lung	A_0	2.00	3.21
	$T_{1/2}$	24.77	60.2
Heart	A_0	4.00	7.71
	$T_{1/2}$	27.2	64.6
Liver	A_0	3.00	5.00
	$T_{1/2}$	29.0	87.4
Spleen	A_0	4.00	5.00
	$T_{1/2}$	24.96	64.9
Kidney	A_0	2.2	3.00
	$T_{1/2}$	41.3	77.7

3.3.4 Urine Activity Biodistributions

Urine biodistribution data was available for patients treated with ^{131}I -CHT-25 and showed considerable variability; this data is shown in Figure 3.8. Individual patient data from patients undergoing repeated therapies is shown in Figure 3.9 and suggests that there was less intra-patient rather than inter-patient variability.

Figure 3.8: Urine Biodistribution Data For All Patients Treated With ^{131}I -CHT-25

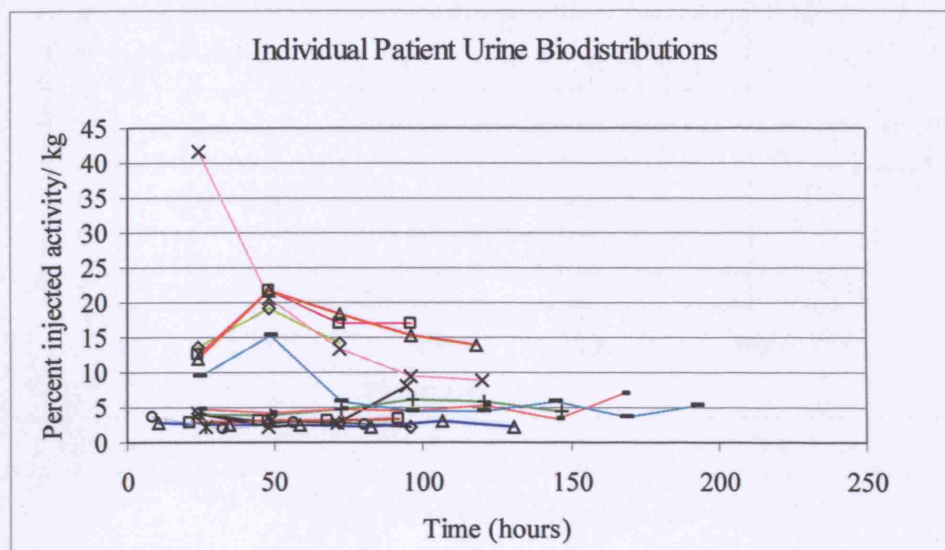
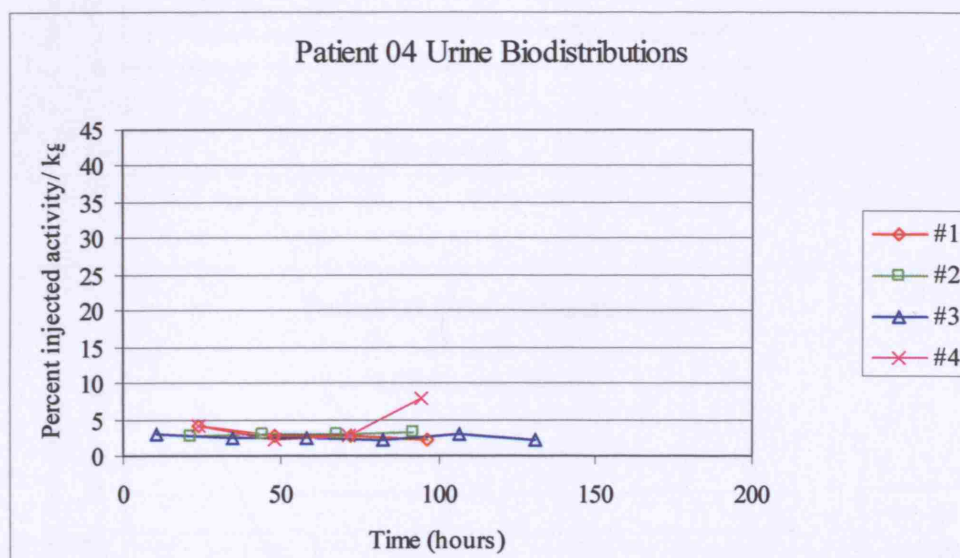
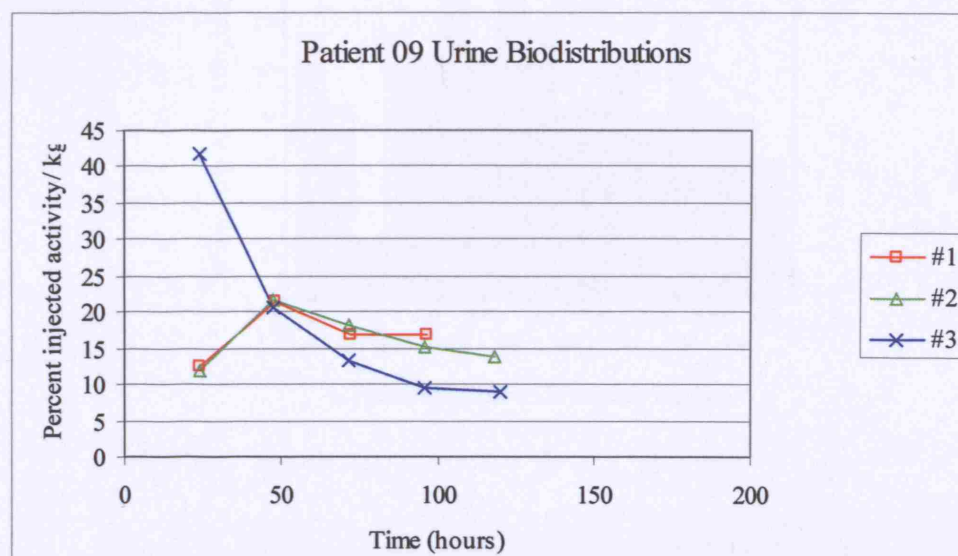
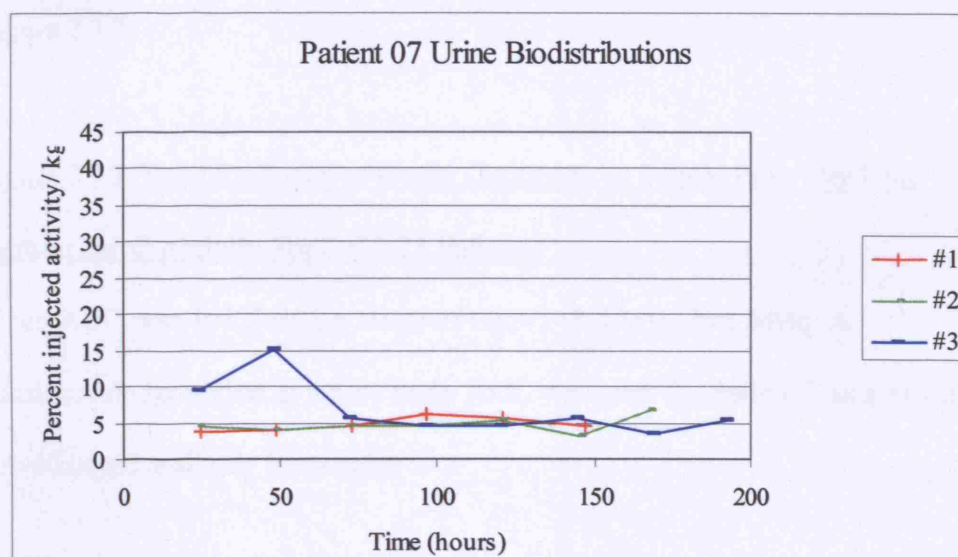
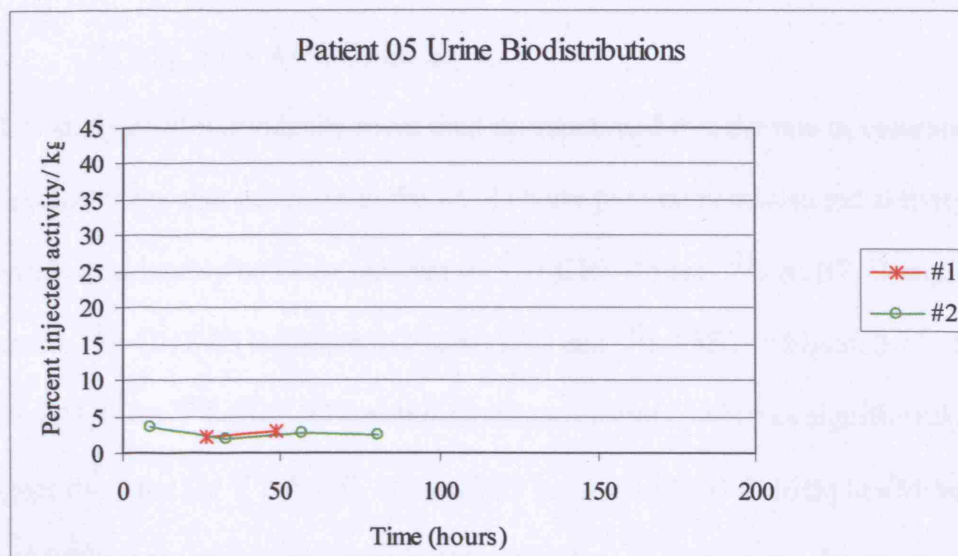


Figure 3.9: Urine Biodistribution Data For Individual Patients Treated With ^{131}I -CHT-25 Undergoing Repeated Therapies.





3.5 Whole Body AUC Estimates

AUC analysis of individual patient data demonstrated that the rate of clearance of radioactivity that occurred in the whole body per unit administered activity varied considerably between patients for ^{131}I -CHT-25 and ^{131}I -A5B7. Complete data for ^{131}I -CHT-25 is shown in Figure 3.10 and ^{131}I -A5B7 in Figure 3.11. The mean AUC for ^{131}I -CHT-25 (excluding data on patient 3#2) was significantly higher than that for ^{131}I -A5B7, being 101.9 compared to 41.3 MBq.hrs/MBq ($p < 0.001$); mean values along with 95% confidence intervals are shown in Figure 3.12.

Figure 3.10: Total Radioactive Decay Occurring In Whole Body Per Unit Administered Activity For CHT-25 Patients.

Mean AUC was 101.9 with a range of 60.3-148.5 MBq.hrs/ MBq. A considerable reduction in whole body AUC was seen for Patient 3 as a result of anti-idiotypic antibody formation.

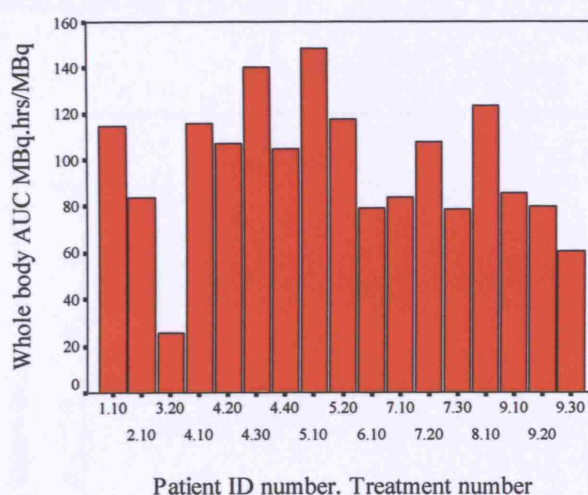


Figure 3.11: Total Radioactive Decay Occurring In Whole Body Per Unit Administered Activity For A5B7 Patients. Mean AUC Was 41.3 with a range of 28.5-55.5 MBq.Hrs/ MBq

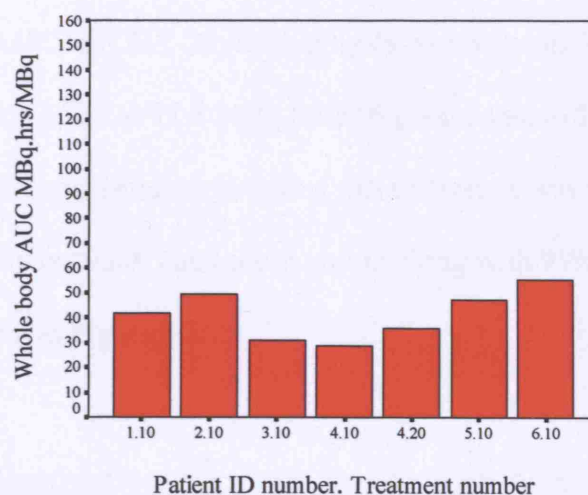
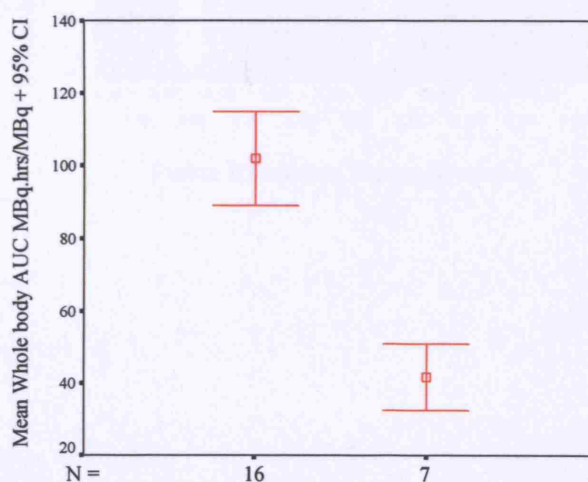


Figure 3.12: Mean Whole Body AUC Per Unit Administered Activity For CHT-25 And A5B7 Patients With 95% Confidence Intervals.

Data from treatment 3#2 was excluded due to a presumed anti-idiotypic response. Mann Whitney U testing revealed a statistically reduced AUC for A5B7 compared with CHT-25 ($p < 0.001$).



3.3.6 Red Marrow AUC estimates

The amount of radioactive decay that occurred in red marrow per unit administered activity varied considerably between patients for both CHT-25 and A5B7. Complete data for CHT-25 is shown in Figure 3.13 and A5B7 in Figure 3.14. The mean AUC for CHT-25 (excluding data on Patients 3#2 and 6) was higher than that for A5B7 at 11.8 MBq.hrs/MBq compared to 3.6 MBq.hrs/MBq. On non-parametric testing this difference was found to be highly significantly ($p < 0.001$) and mean values along with 95% confidence intervals are shown in Figure 3.15.

Figure 3.13: Radioactive Decay Occurring In Blood Per Administered Activity For CHT-25 Patients.

Mean AUC was 20.95 with a range of 2.11-49.01 MBq.hrs/ MBq.

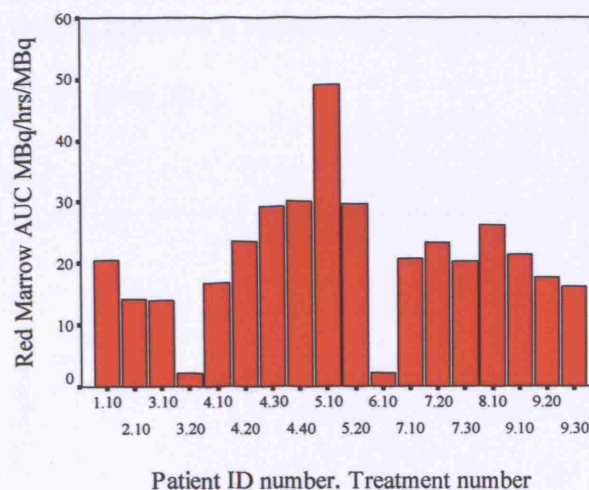


Figure 3.14: Radioactive Decay Occurring In Red Marrow Per Administered Activity For A5B7 Patients.

Mean AUC was 7.06 with a range of 3.86-10.46 MBq.hrs/ MBq.

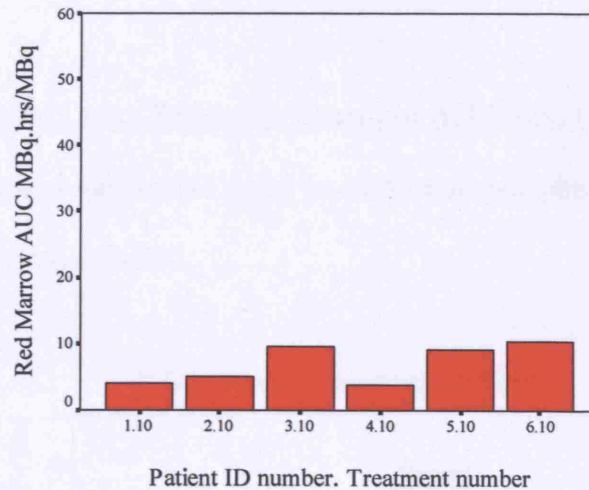
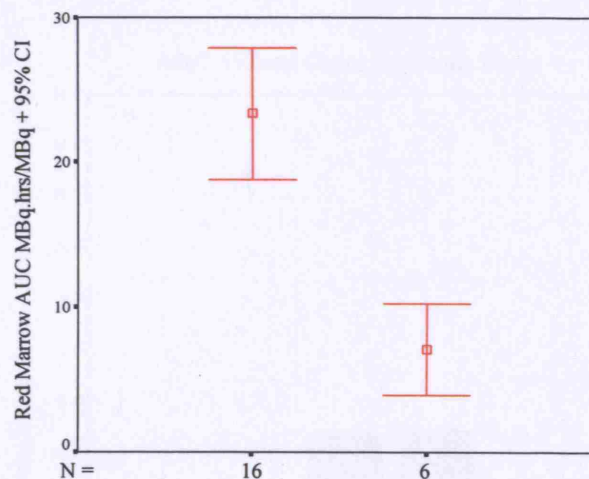


Figure 3.15: Mean Red Marrow AUC For CHT-25 And A5B7 Patients With 95% Confidence Intervals.

Mann Whitney U testing revealed a statistically reduced AUC for A5B7 compared with CHT-25 ($p < 0.001$).

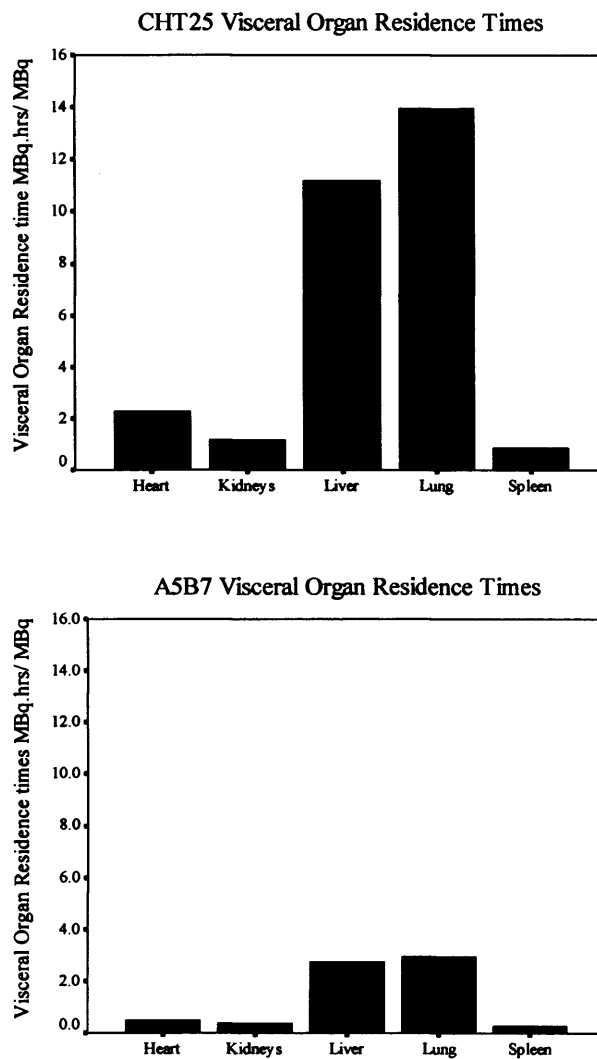


3.3.7 Visceral Organ AUC Estimates

Whole organ AUC estimates for both population groups were modelled exponentially and are shown in Figure 3.16. For all organs a higher residence time was observed for CHT-25 than A5B7.

Figure 3.16: Visceral Organ Residence Times For A5B7 And CHT-25.

This reveals increased radioactive decay in each organ per administered activity for CHT-25 compared to A5B7.



3.3.8 MIRD Absorbed Dose Estimates To Normal Tissues

When analysed by MIRD the varied pharmacokinetic profiles for the two antibodies translate into radiation-absorbed doses 2-4 times higher per equivalent administered activity for CHT-25 compared to A5B7, depending upon the organ of interest. These are shown in Table 3.5.

Table 3.5: Normal Organ Absorbed Doses Estimated According To The MIRD Formulary Calculated Using Olinda Software Giving Total Absorbed Dose In mGy/ Mbq.

Organ	A5B7	CHT-25
Lung	0.43	1.85
Heart	0.24	1.06
Liver	0.24	0.96
Spleen	0.21	0.70
Kidneys	0.21	0.63
Marrow	0.44	1.18

Estimated radiation absorbed doses for ‘typical’ 1.7m² subjects for A5B7 and CHT-25 are shown in Tables 3.6 and 3.7 respectively

Table 3.6: Estimated Doses To Normal Organs For A Typical 1.7 M² Subject Using A5B7 Antibody

Administered Activity MBq/m²	Radiation absorbed Dose (Gy)					
	Lung	Heart	Liver	Spleen	Kidney	Marrow
370	0.27	0.15	0.15	0.13	0.13	0.27
740	0.54	0.3	0.31	0.26	0.26	0.55
1480	1.08	0.6	0.61	0.52	0.52	1.09
2220	1.62	0.9	0.92	0.78	0.78	1.64
2960	2.16	1.20	1.22	1.04	1.04	2.19

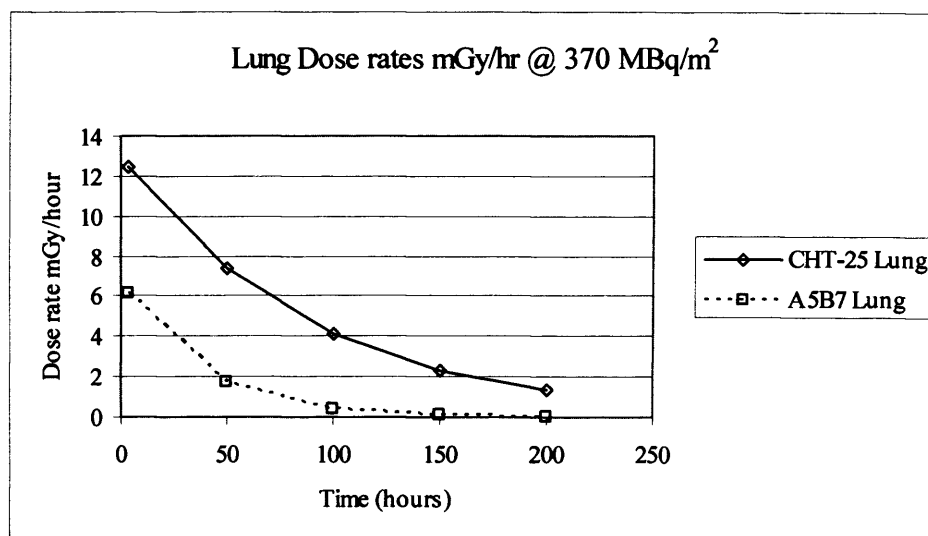
Table 3.7: Estimated Doses To Normal Organs For A Typical 1.7 M² Subject using CHT-25 Antibody

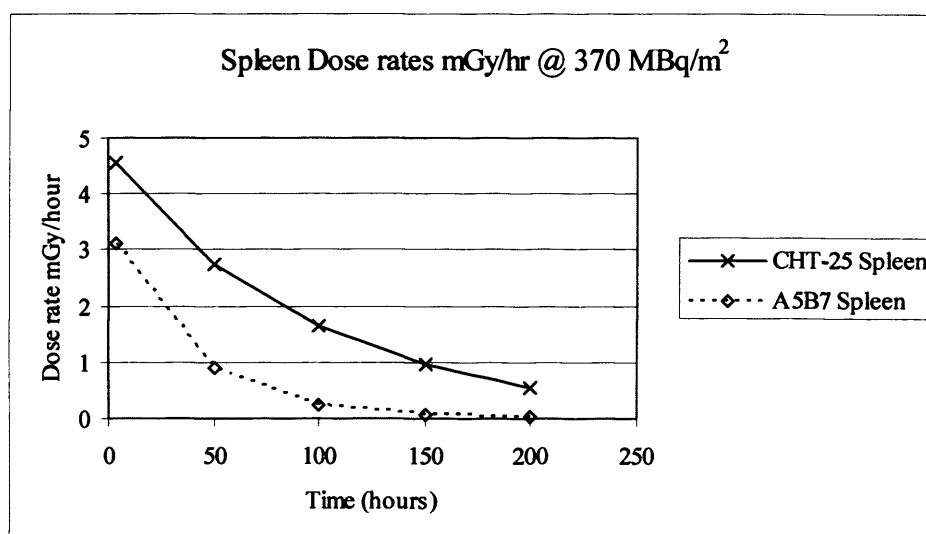
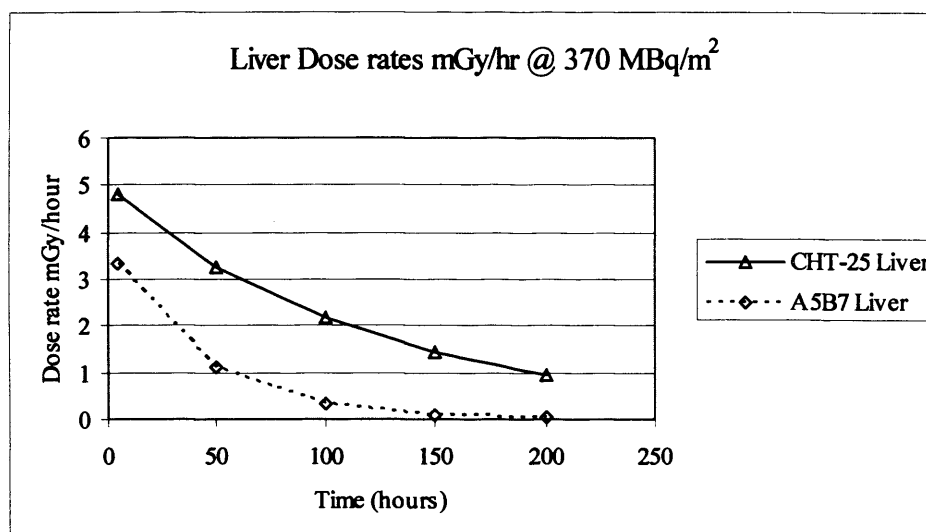
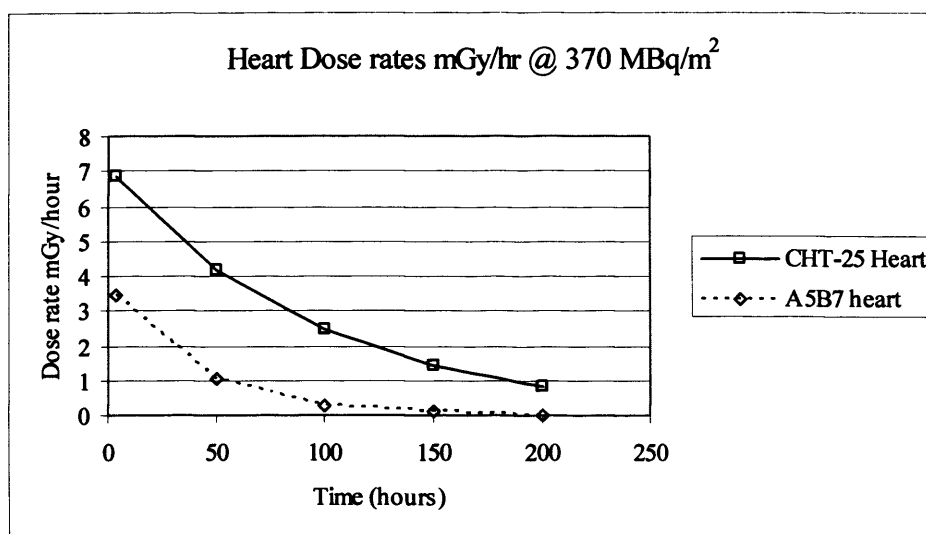
Administered Activity MBq/m ²	Radiation absorbed Dose (Gy)					
	Lung	Heart	Liver	Spleen	Kidney	Marrow
370	1.16	0.67	0.6	0.44	0.39	0.74
740	2.33	1.33	1.2	0.88	0.79	1.48
1480	4.65	2.67	2.41	1.77	1.58	2.97
2220	6.98	4.00	3.61	2.65	2.37	4.45
2960	9.31	5.33	4.82	3.54	3.16	5.94

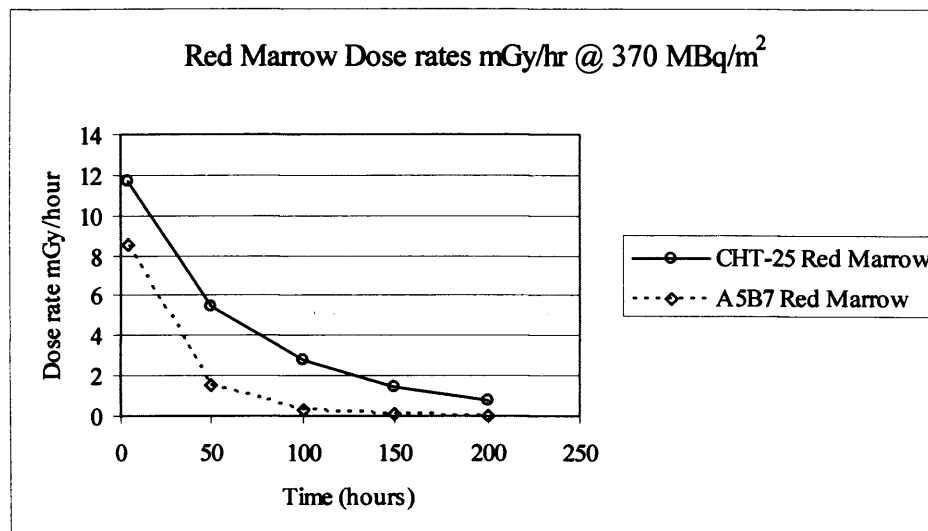
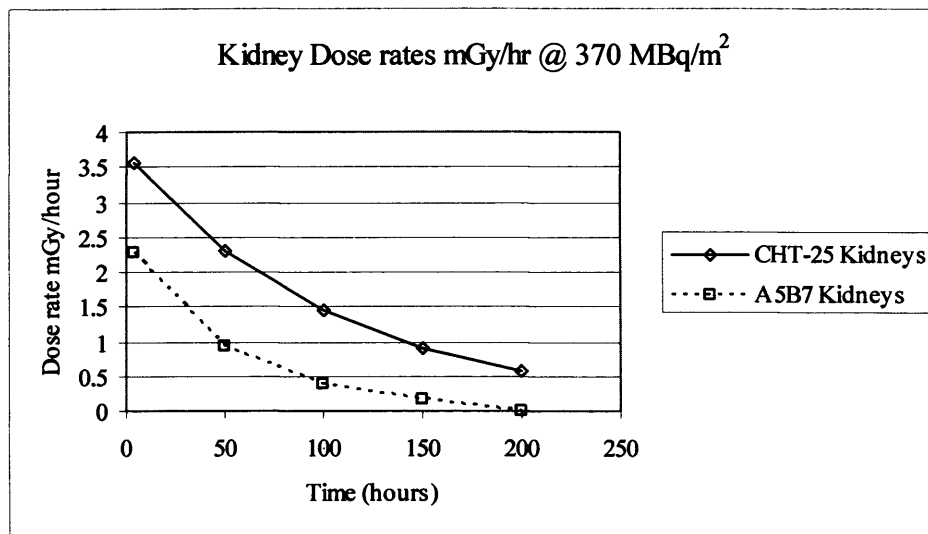
3.3.9 MIRD Dose Rate Estimates To Normal Tissues

Using MIRD, an estimate of normal tissue absorbed dose rates have also been made. The absorbed dose rate in normal tissues increased with administered activity, but for any given administered activity dose rates were higher for CHT-25 than A5B7 and are shown in Figure 3.16.

Figure 3.16: Absorbed Dose Rate Estimates For A5B7 And CHT-25 For 740 Mbq/M² Dose Level And Assuming a 1.7 m² Subject Revealing Higher Absorbed Dose Rates For CHT-25 In All Organs.

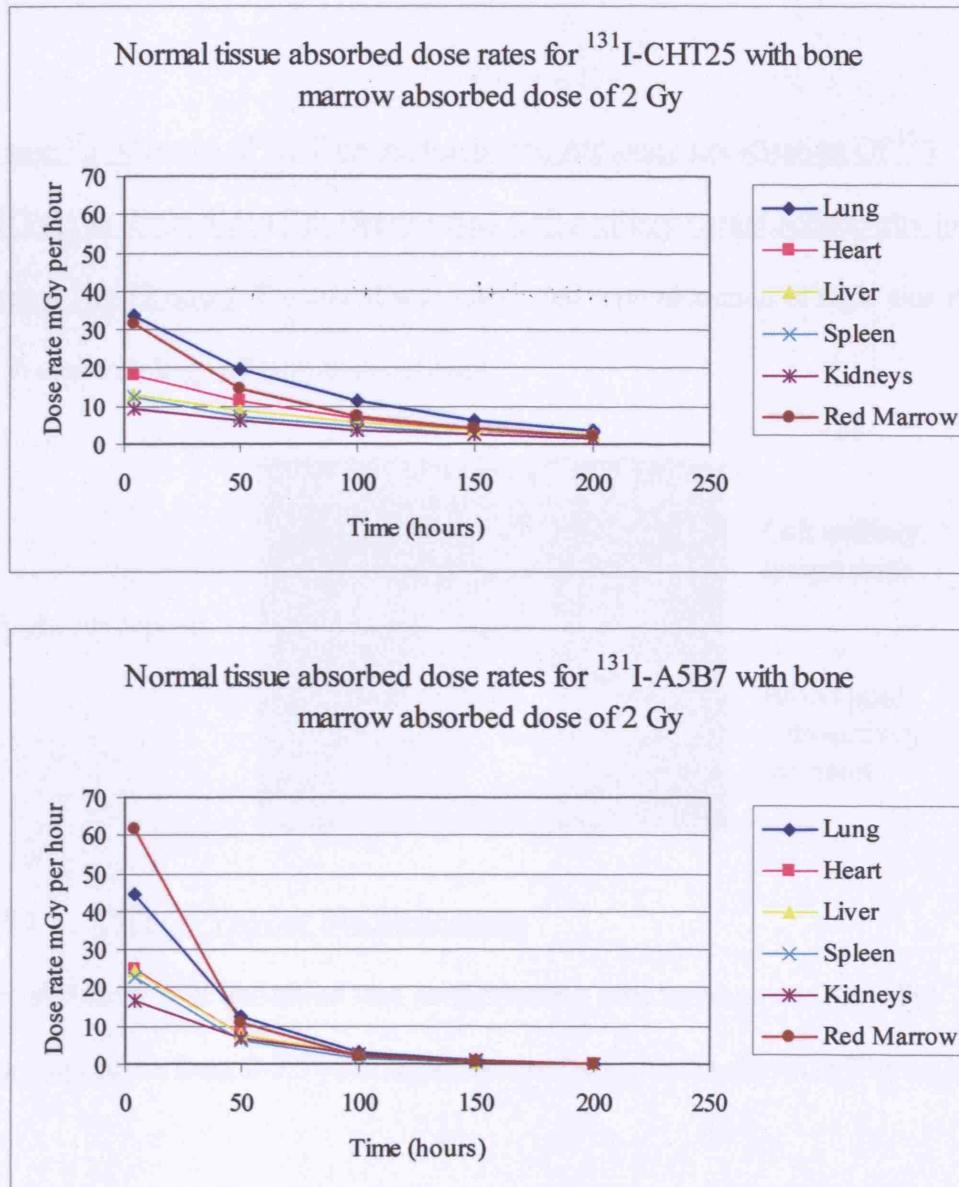






However, since in practical terms for non-myeloablative RIT the maximum doses of activity that can be delivered by each antibody will depend upon the bone marrow dose, I have compared the dose rates that would be achieved by administering both antibodies in order to deliver an identical bone marrow dose. Using the MIRD calculations above, it can be seen that the activity to deliver 2 Gy to bone marrow for ¹³¹I-CHT-25 is 1694 MBq and for ¹³¹I-A5B7 is 4545 MBq. Results are shown in Figure 3.17 and demonstrate that the longer half-life of ¹³¹I-CHT-25 means it will deliver a lower dose rate of radiation to normal tissues but over a longer time course than ¹³¹I-A5B7.

Figure 3.17: Normal Organ Dose Rates Following Administration Of Activity To Deliver A Fixed Dose of 2 Gy To Bone Marrow



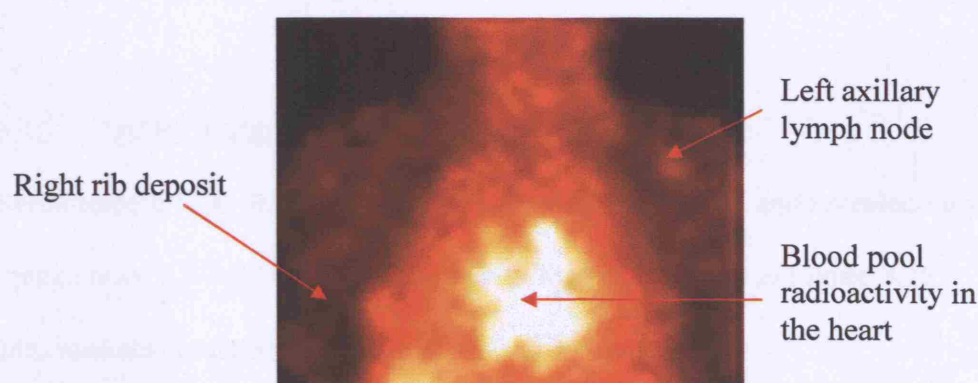
3.4 Results II: Tumour Biodistributions and Dosimetry

Evidence of tumour localisation has been obtained in all treatments using ^{131}I -A5B7, with the majority of tumour deposits being secondary liver metastases.

For

^{131}I -CHT-25, evidence of tumour localisation was seen in 12 out of 18 treatments, mainly into malignant lymphadenopathy, and was greater than 3% of injected activity/kg in 9 of these. Illustrations of ^{131}I -CHT-25 uptake into a right sided rib deposit and left sided axillary lymphadenopathy are shown in Image 3.1.

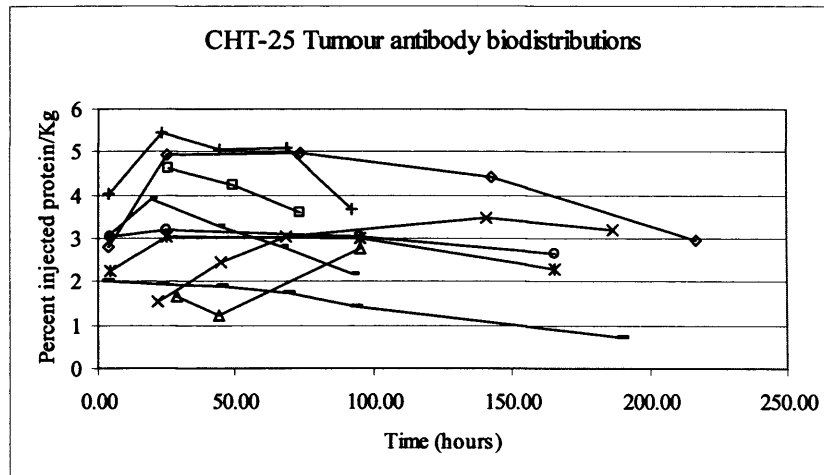
Image 3.1: Coronal SPECT Image Revealing Antibody Localisation Of ^{131}I CHT-25 In Right Sided Rib Deposit And Left Axillary Lymphadenopathy In Patient 7 at 72 hours. Treatment was associated with reduction of right side rib pain and shrinkage of lymphadenopathy.



3.4.1 CHT-25 Tumour Biodistributions

Serial tumour ROI definition was performed for nine tumours and revealed varying uptake from 2-5.5 % of injected activity/kg and is shown in Figure 3.18

Figure 3.18: ^{131}I -CHT-25 Tumour Antibody Biodistributions Revealing Uptake Values Of 2-5.5% Of Injected Protein With Peak Accumulation Generally Being Seen 24-48 Hours Following Administration Of Antibody.



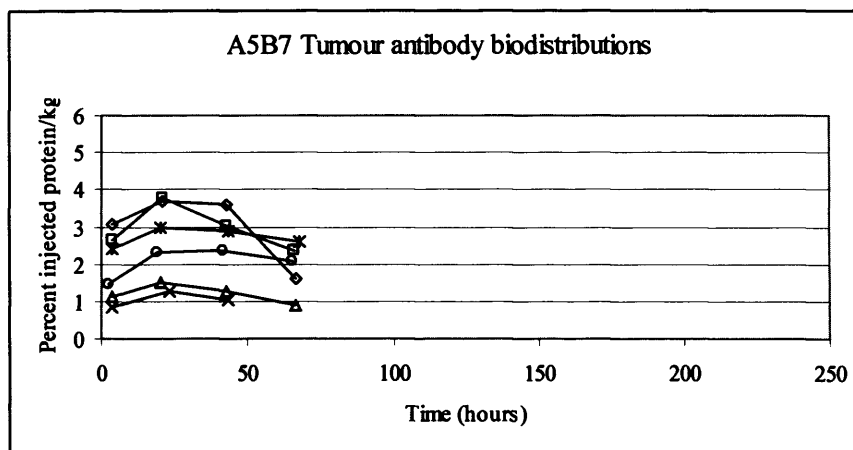
3.4.2 A5B7 Tumour Biodistributions

Serial tumour ROI definition was performed for 6 tumours and revealed varying uptake from 1.3-3.8 % of injected activity/kg and is shown in Figure 3.19.

Maximal accumulation was seen at around 24 hours.

Figure 3.19: ^{131}I A5B7 Tumour Antibody Biodistributions For 6 Tumours.

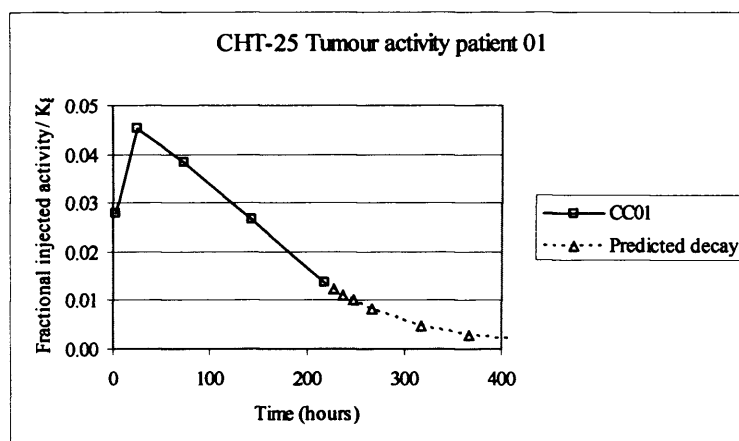
Most were liver metastatic deposits.

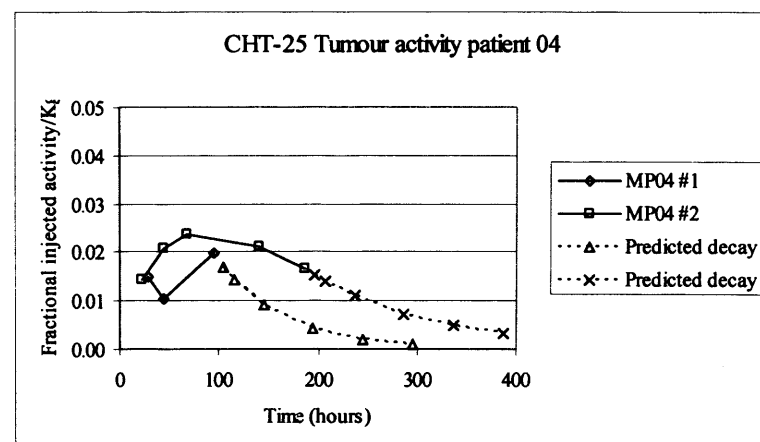
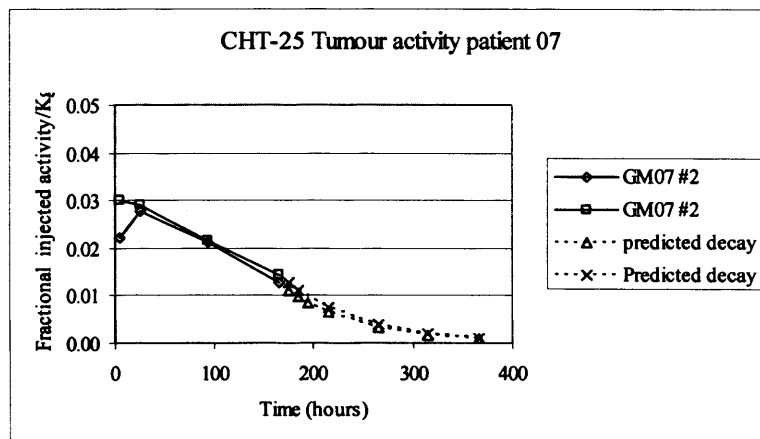
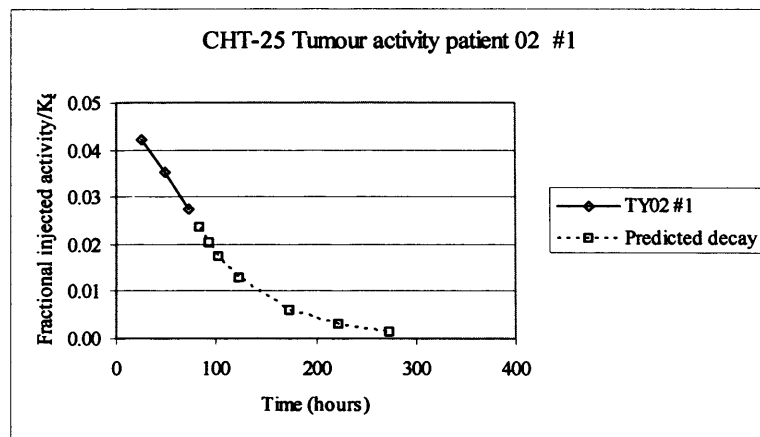


3.4.3 AUC Estimate For Tumours

Measured data points for CHT-25 and A5B7 are shown along with their estimated terminal decay values in Figures 3.20 and 3.21.

Figure 3.20: CHT-25 Tumour Activity Biodistributions With Measured Values Followed By Estimated Terminal Clearance Times Assuming Terminal Blood Biodistribution Kinetics.





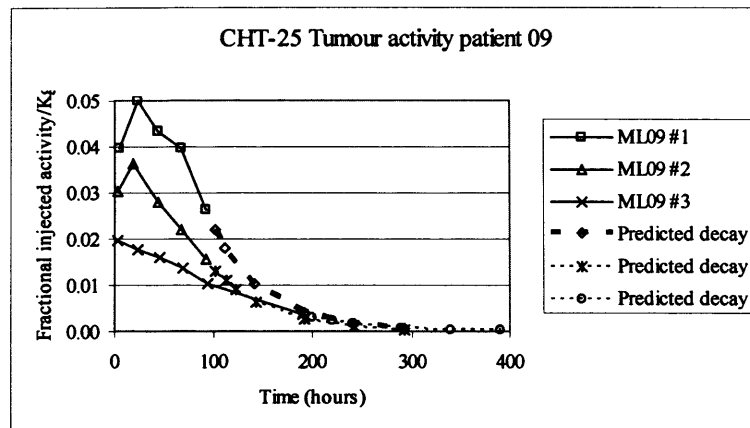
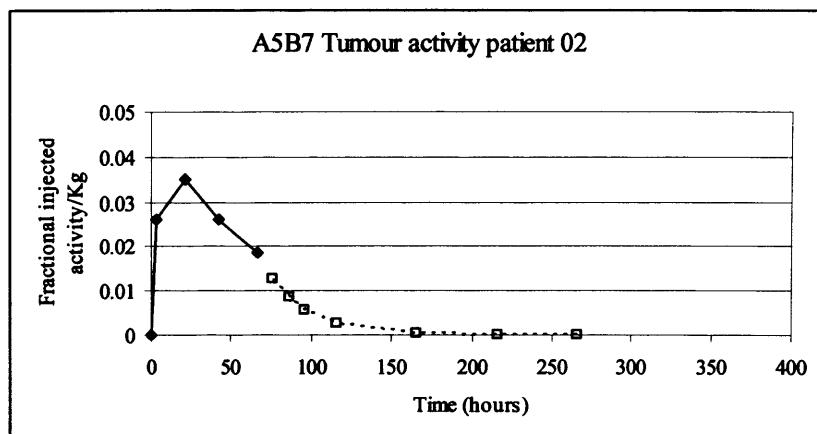
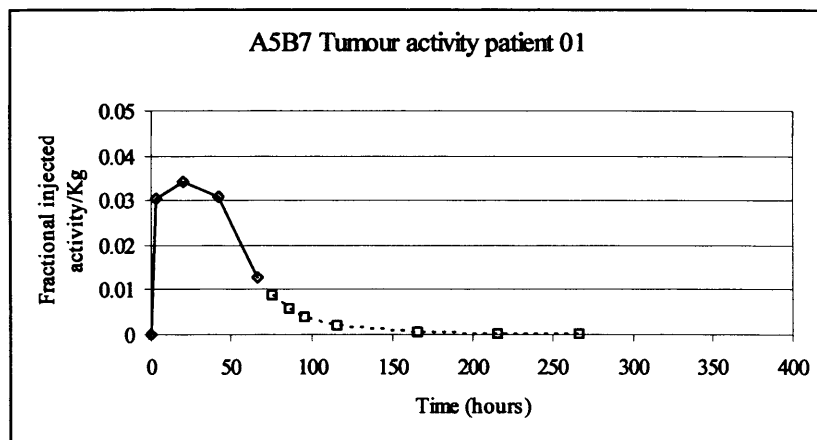
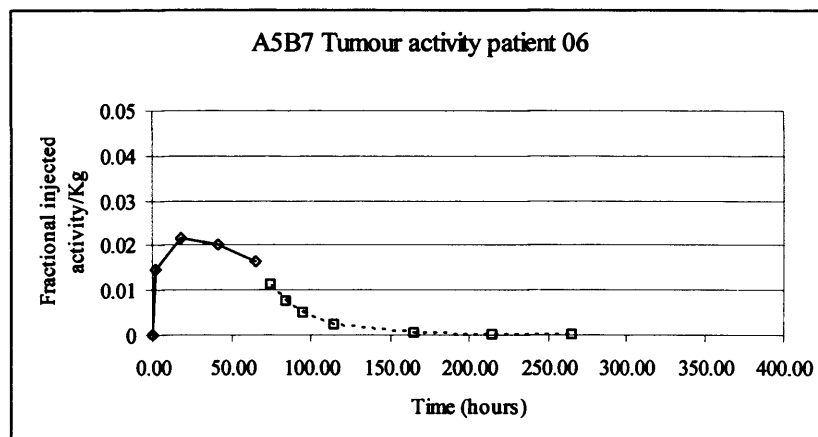
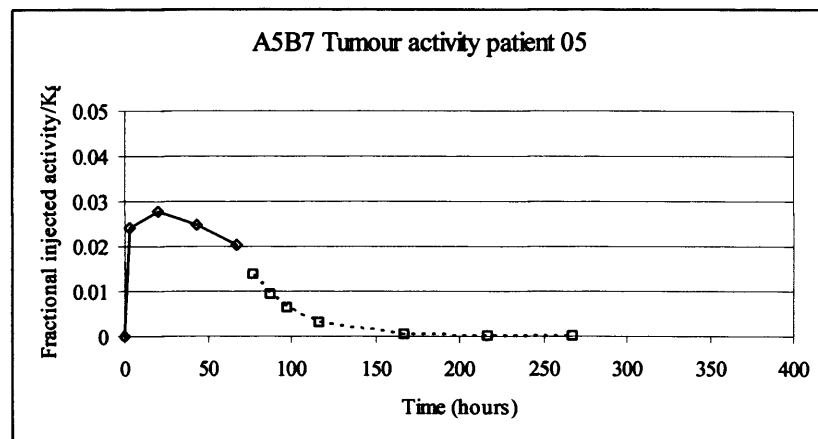
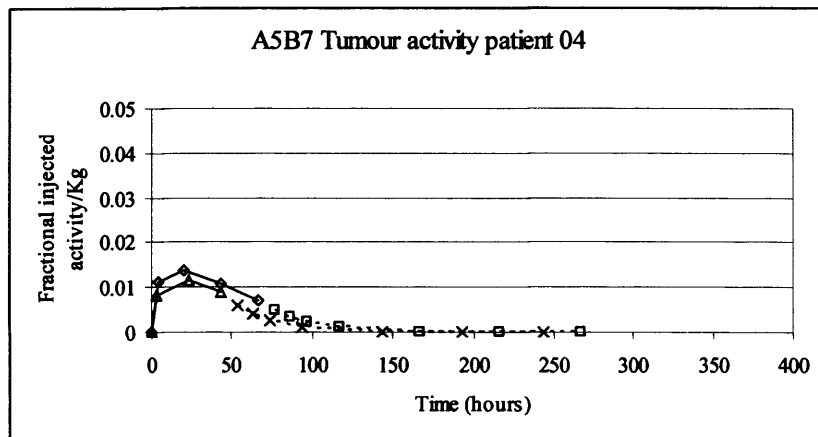


Figure 3.21: A5B7 Tumour Activity Biodistributions With Measured Values Followed By Estimated Terminal Clearance Times Assuming Terminal Blood Biodistribution Kinetics.

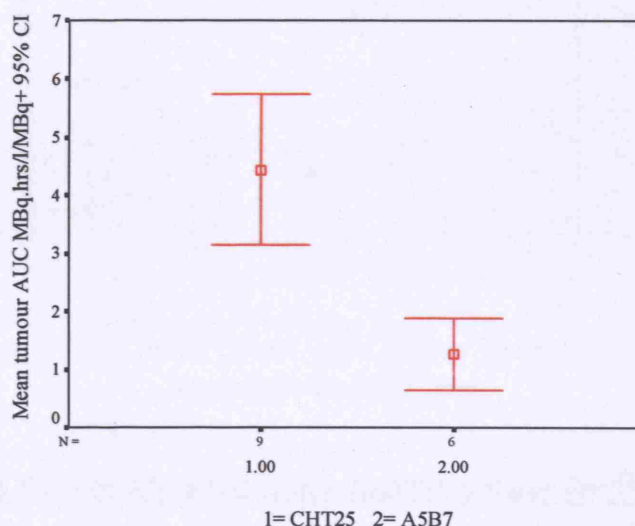




Per unit administered activity, ^{131}I -CHT-25 delivered a higher amount of radiation to tumour sites than ^{131}I -A5B7. Mean tumour AUC per MBq was 4.44 MBq.hrs/l/MBq and 1.27 MBq.hrs/l/MBq for ^{131}I -CHT-25 and ^{131}I -A5B7 respectively. This data is shown in Figure 3.22 along with 95% confidence intervals. This difference was statistically significant ($p=0.01$).

Figure 3.22: Mean Tumour AUC Values Per Unit Administered Activity For A5B7 And CHT-25 With 95% Confidence Intervals.

The mean AUC for A5B7 was 1.26 MBq.hrs/l/MBq and the mean AUC for CHT-25 was 4.44 MBq.hrs/l/MBq.



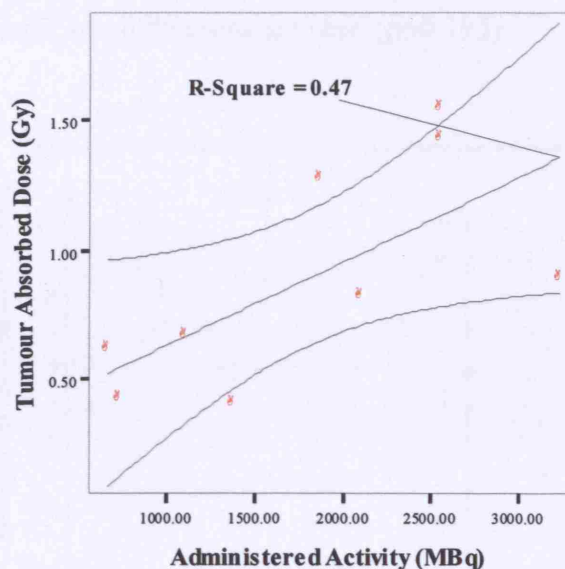
3.4.4 MIRD Tumour Absorbed Dose Estimates

These were estimated by entering the total tumour residence times into the Olinda spherical model using the appropriate tumour mass as estimated from CT scans.

For ^{131}I -A5B7 where the administered activity per surface area was constant for different patients at 1800 MBq/m^2 , tumour absorbed doses showed considerable variability, with a mean tumour absorbed dose of 0.57 Gy (range 0.21-0.95 Gy).

For ^{131}I -CHT-25 where a number of different doses were administered, the mean tumour absorbed dose was 0.89 Gy and ranged from 0.4 -1.54 Gy. For this group of patients a significant correlation was seen between tumour absorbed dose and total administered activity when analysed by linear regression. This is shown in Figure 3.23 ($p=0.043$).

Figure 3.23: Correlation Between Administered Activity And Tumour Absorbed Dose Analysed By Linear Regression For ^{131}I -CHT-25 With 95% CI



3.4.5 MIRD Tumour Absorbed Doses And Dose Rates For ^{131}I -CHT-25 in Comparison To ^{131}I -A5B7

For a fixed activity to deliver a 2 Gy dose to the bone marrow, the tumours examined would have a slightly higher mean total absorbed dose using ^{131}I -CHT-25 compared to ^{131}I -A5B7, but this is not statistically significant ($p=0.195$). In a similar fashion to normal tissue however the maximal dose rates in tumour by ^{131}I -CHT-25 would also be reduced in comparison to ^{131}I -A5B7, although the overall treatment times would again be longer. This data is shown in Figures 3.24 and 3.25

Figure 3.24: Predicted Tumour Total Absorbed Dose Estimated for ^{131}I -CHT-25 and ^{131}I -A5B7 when Administering an Activity that Delivers 2 Gy To The Red Marrow. No significant differences are seen ($p=0.195$).

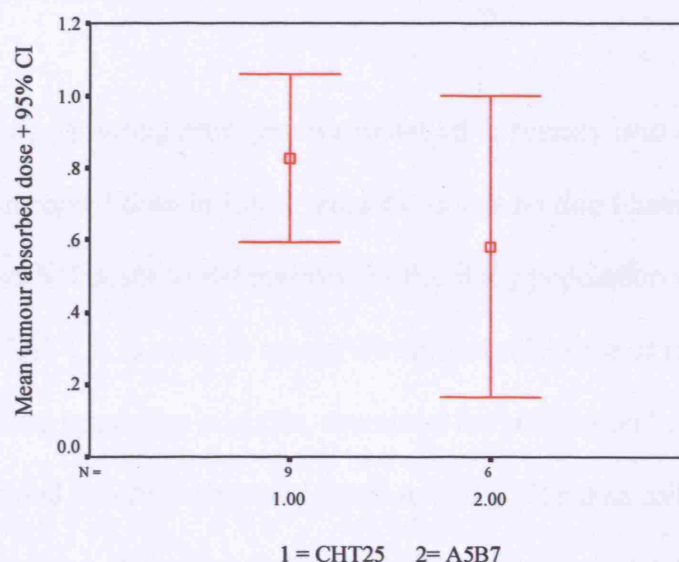
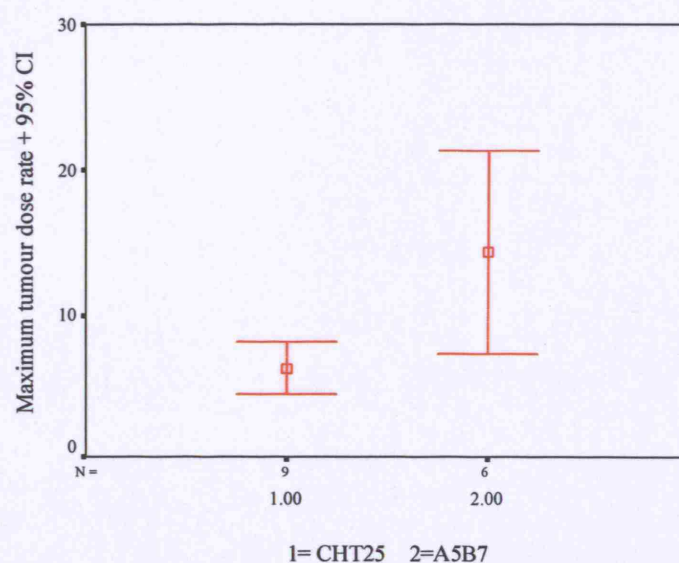


Figure 3.25: Mean Maximal Tumour Absorbed Dose Rates For ^{131}I -CHT-25 And ^{131}I -A5B7 For An Absorbed Bone Marrow Dose Of 2 Gy.

The maximal dose rate achieved by ^{131}I -A5B7 was significantly higher for than ^{131}I -CHT-25 ($p=0.01$).

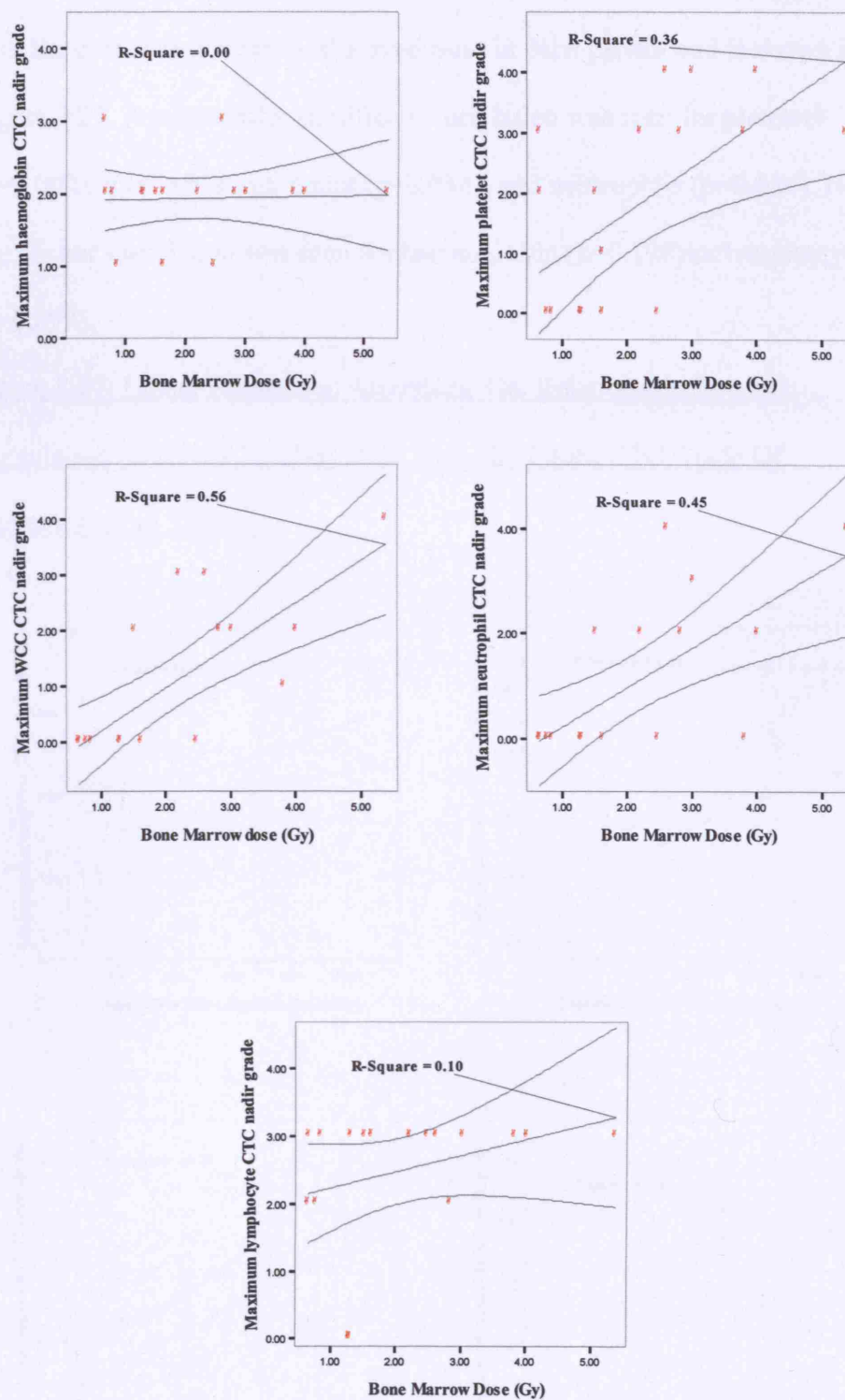


3.5 Ability Of Population Based Dosimetry And Individual Biodistribution Data To Predict Observed Haematological Toxicity

3.5.1 Correlation Between MIRD Absorbed Dose Estimates And Observed Haematological Toxicity

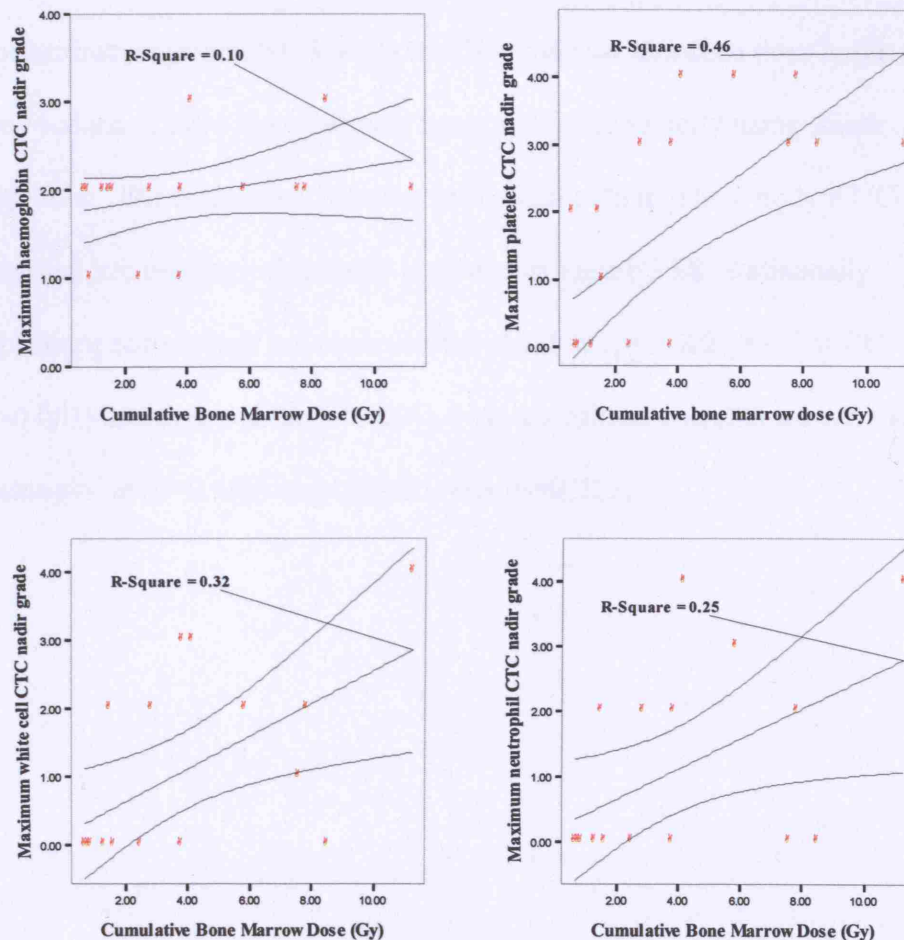
The purpose of performing retrospective radiation dosimetry is to allow a prediction of absorbed dose in future patients. In this section I have estimated the MIRD absorbed doses to red marrow for the study population which received ^{131}I -CHT-25. In order to assess the clinical relevance of these estimates I have then, using regression analysis, examined the correlation between marrow absorbed dose and observed haematological toxicity. The data following each single administration of antibody is shown in Figure 3.26. A statistically significant correlation between marrow absorbed dose and CTC toxicity grade was observed for platelets ($p=0.009$), total white cell count ($p=0.0009$) and neutrophils ($p=0.002$). However, no significant association was evident for haemoglobin ($p=0.890$) or lymphocytes ($p=0.394$).

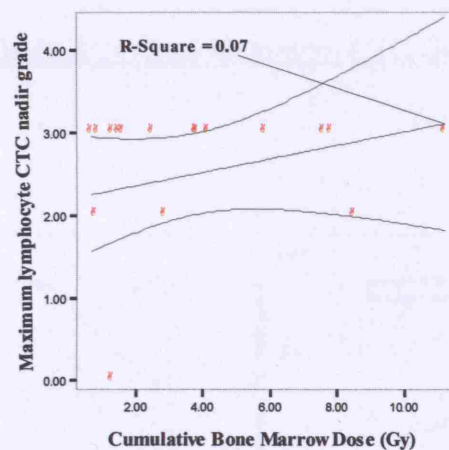
Figure 3.26: Linear Regression Analysing The Relationship Between Marrow Absorbed Dose And Maximum CTC Grade Of Haematological Toxicity.



In addition, since haematological toxicity is likely to be related to cumulative marrow absorbed dose and many of the patients in this study also underwent multiple therapies, observed haematological toxicity has also been correlated with the cumulative marrow absorbed dose in each patient and is shown in Figure 3.27. A statistically significant correlation was seen for platelets ($p=0.002$), total white cell count ($p=0.014$), and neutrophils ($p=0.034$). No significant association was seen for haemoglobin ($p=0.198$) or lymphocytes ($p=0.297$).

Figure 3.27: Linear Regression Analysing The Relationship Between Cumulative Marrow Absorbed Dose And Maximum CTC Grade Of Haematological Toxicity.

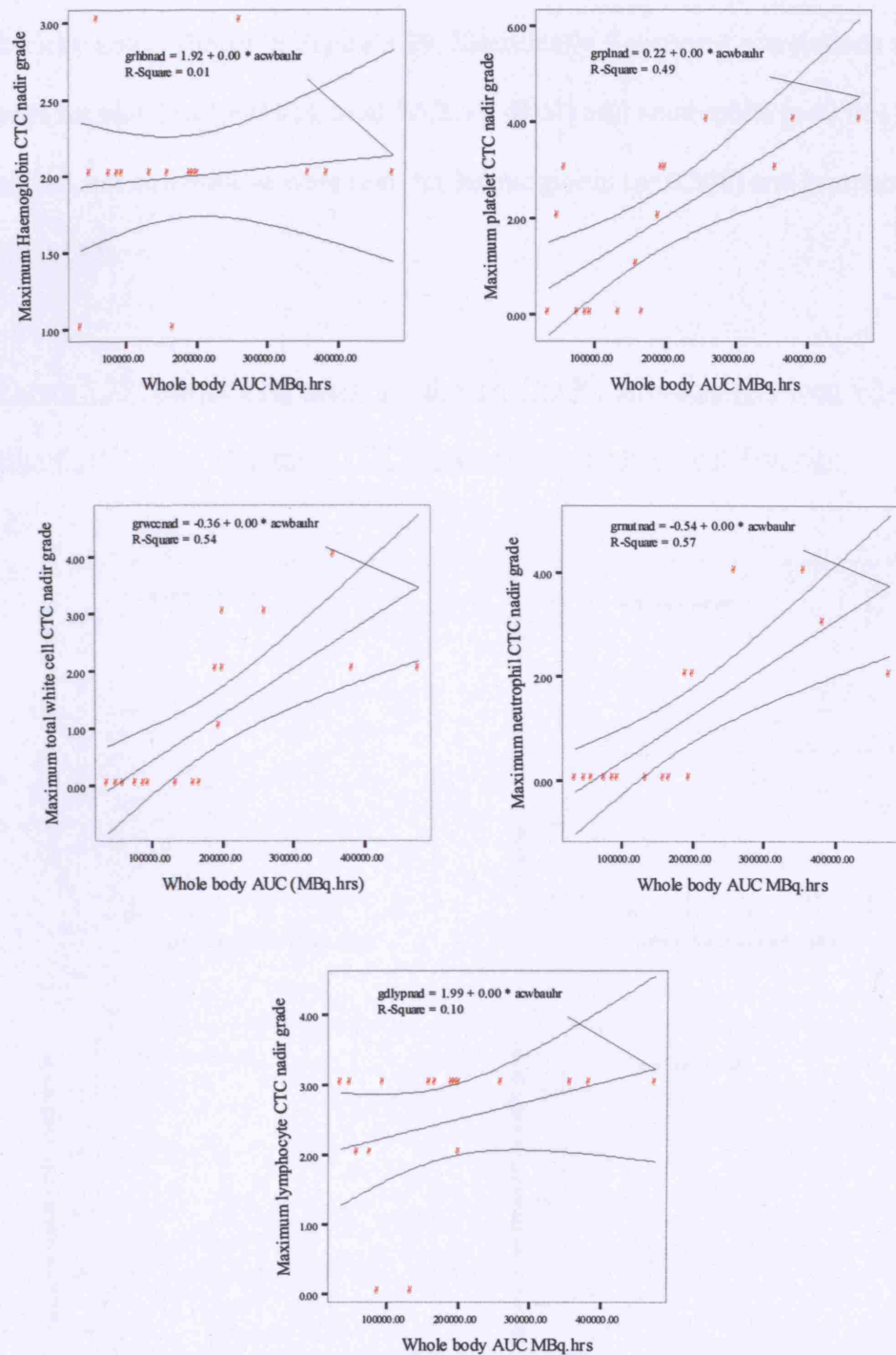




3.5.2 Correlation Between Individual Patient Whole Body AUC And Observed Haematological Toxicity

Given that there appears to be significant intra-patient variability in activity biodistributions, I have examined the correlation between simple but individual biodistribution parameters known to affect marrow absorbed dose in the next two sections. I have also observed haematological toxicity using linear regression. The correlation between individual patient whole body AUC and observed haematological toxicity is shown in Figure 3.28. Statistically significant correlations are observed for platelets ($p=0.002$), total WCC ($p=0.001$) and neutrophils ($p<0.001$). Non significant correlations were seen for haemoglobin ($p=0.660$) and lymphocytes ($p=0.205$).

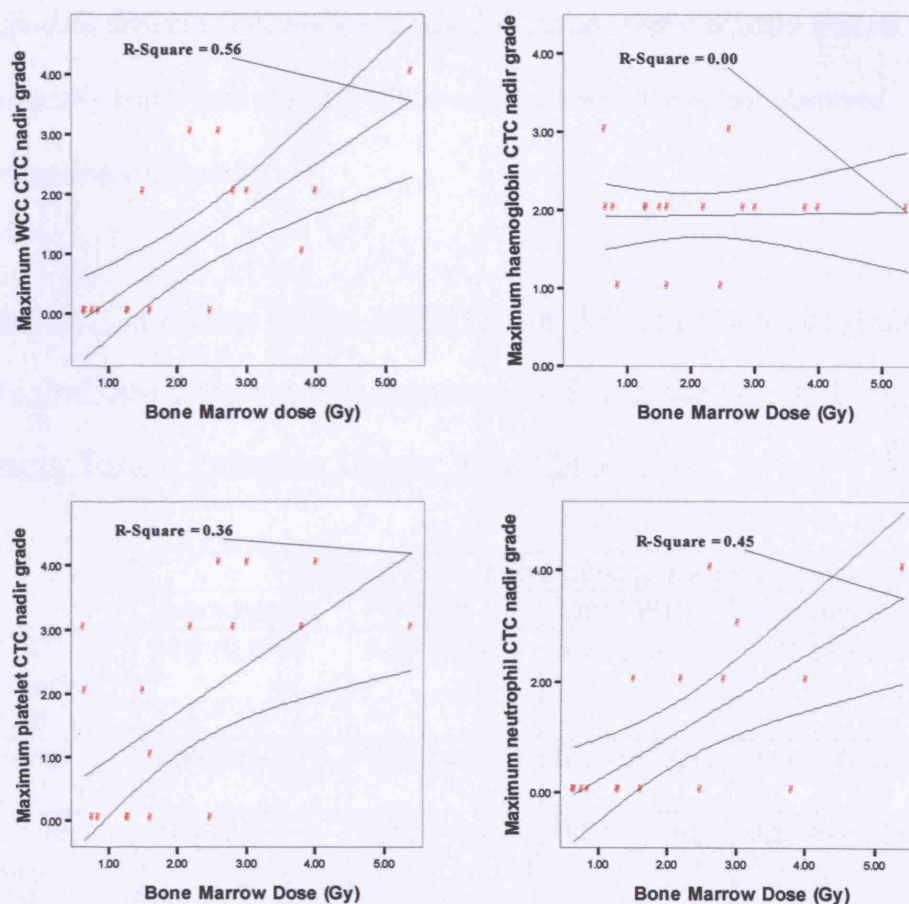
Figure 3.28: Linear Regression Analysing The Relationship Between Cumulative Whole Body AUC And Maximum CTC Grade Of Haematological Toxicity

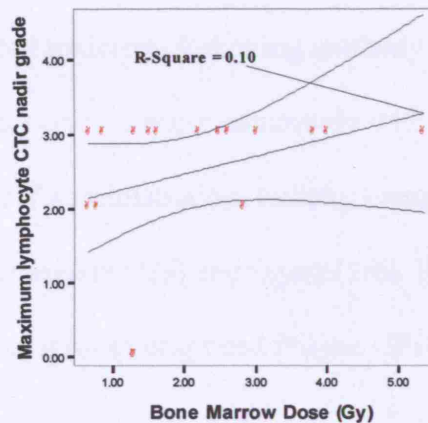


3.5.3 Correlation Between Individual Patient Blood AUC And Observed Haematological Toxicity.

In this section linear regression has been performed to look for evidence of a correlation between individual patient blood AUC and observed haematological toxicity and is shown in Figure 3.29. Statistically significant correlations were seen for platelets ($p<0.01$), total WCC ($p<0.01$) and neutrophils ($p<0.001$). Non significant correlations were seen for haemoglobin ($p=0.308$) and lymphocytes ($p=0.145$).

Figure 3.29: Linear Regression Analysing The Relationship Between Whole Blood AUC And Maximum CTC Grade Of Haematological Toxicity.





3.5.4 Comparison Of Population Derived MIRD Dose Estimates And Individual Biodistribution Parameters To Predict Haematological Toxicity

Comparing the ability of MIRD dose estimates to that of individual patient biodistribution data, it is seen that, despite the simple nature of the latter, individual patient whole body and blood AUC correlated equally well or marginally better than absorbed dose estimates with the actual observed haematological toxicity.

Table 3.8: Comparison Of The Ability Of The Population Derived MIRD Absorbed Dose Estimates With Individual Biodistribution Data To Estimate Marrow Toxicity Following Therapy With ^{131}I -CHT-25.

	Pearson Correlation value (r^2) with associated p-values				
	Haemoglobin	Platelets	Total WCC	Neutrophils	Lymphocytes
MIRD Absorbed Dose	0.00 (0.890)	0.35 (<0.01)	0.56 (<0.01)	0.45 (<0.01)	0.10 (0.40)
Blood AUC	0.06 (0.308)	0.58 (<0.01)	0.69 (<0.01)	0.68 (<0.01)	0.13 (0.15)
Whole Body AUC	0.01 (0.660)	0.49 (<0.01)	0.54 (<0.01)	0.57 (<0.01)	0.10 (0.21)

3.6 Immunogenicity of ^{131}I -CHT-25 and ^{131}I -A5B7

Acute immune mediated toxicities following antibody administration were noted for both antibodies but occurred more commonly with ^{131}I -A5B7 than ^{131}I -CHT-25. Following ^{131}I -A5B7 administration, toxicity comprised fever (4/6), tachycardia 2/6), hypertension (1/6) and rigors (1/6). Following ^{131}I -CHT-25 administration, acute toxicities comprised fatigue (3/18), pyrexia (2/18) and nausea (1/18).

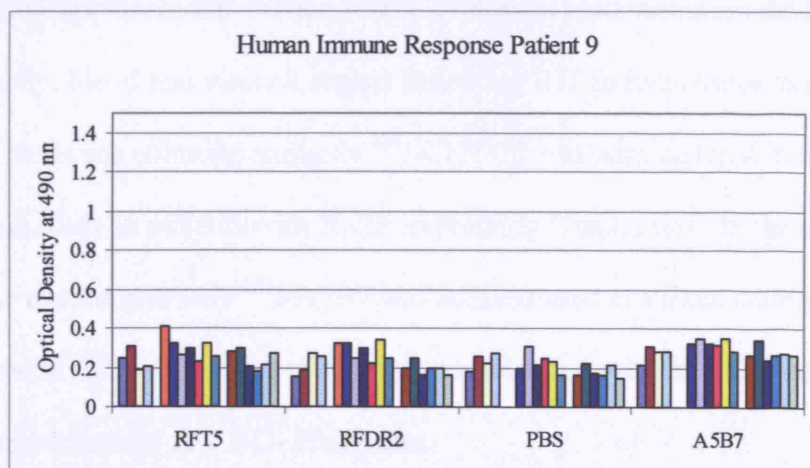
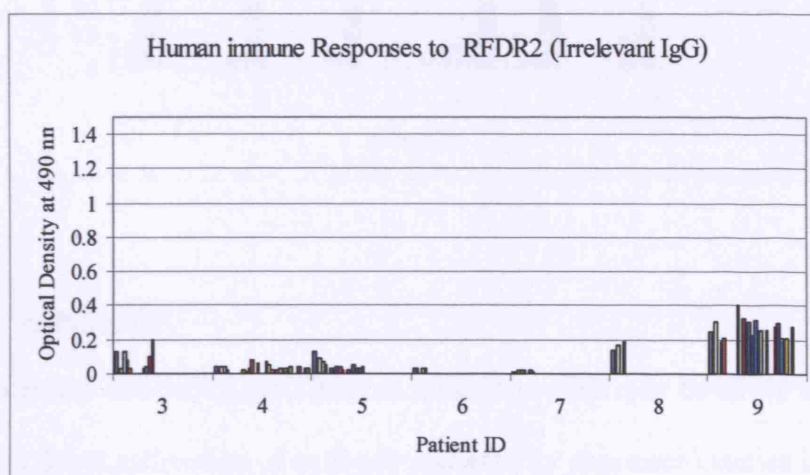
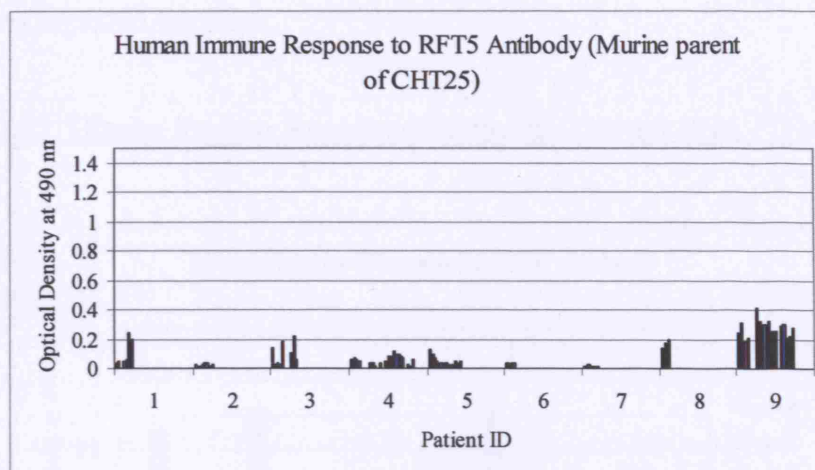
Immunogenicity of ^{131}I -CHT-25 and ^{131}I -A5B7 was assessed using ELISA. With regard to ^{131}I -CHT-25, since the human component of the chimeric antibody would always be picked up by the anti-human antibody used in the detection layer and therefore is non-discriminatory, the human anti-mouse response was assessed using the murine parent antibody RFT5 with RFDR2, a mouse antibody of the same IgG2a class used as an irrelevant control.

The HAMA response to RFT5 and irrelevant control is shown below in Figure 3.30. No convincing evidence of HAMA formation was seen in any of the patients, though the altered pharmacokinetics seen in Patient 3 following antibody administration could be explained by an anti-idiotypic response. Patient 9 was positive to both irrelevant control antibody and PBS at baseline and this fell with therapy, suggesting non-specific antibody binding rather than a true antibody response.

Figure 3.30: Human Immune Responses Following Therapy With ^{131}I -CHT-25

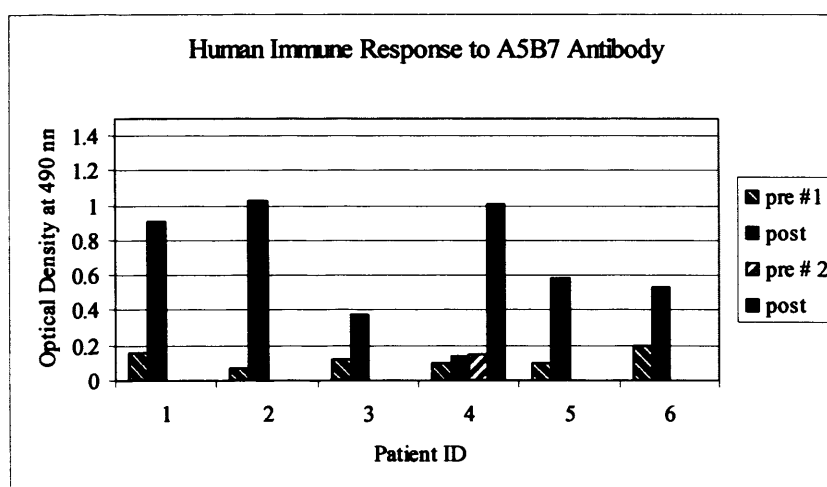
Revealing Low Immunogenicity Even Upon Repeated Exposure.

No documented HAMA responses have been seen to date.



By contrast, all patients treated with ^{131}I -A5B7, with the exception of Patient 4, developed a positive antibody response to ^{131}I -A5B7 following a single antibody exposure. Patient 4 subsequently developed a positive HAMA response following repeated exposure.

Figure 3.31: Human Immune Responses Following Therapy With ^{131}I -A5B7.



3.7 Discussion

Radioiodinated antibodies emit gamma radiation which may be easily measured to allow a direct estimation of antibody and activity pharmacokinetics in normal tissues and tumour. In this section I have compared biodistribution data from whole body, blood and visceral organs following RIT in two clinical studies. In the first study the chimeric antibody ^{131}I -CHT-25 was administered in a dose escalation study to patients with IL-2R expressing lymphomas. In the second study the murine antibody ^{131}I -A5B7 was administered at a fixed dose to patients with CEA expressing tumours in combination with the anti-vascular drug Combretastatin A-4 3-O- Phosphate.

Chimeric antibodies with reduced mouse protein are reported to have lower immunogenicity in comparison to murine ones. In this analysis, data is available following 18 administrations of ^{131}I -CHT-25 to 9 patients with IL-2R expressing lymphomas and in this group no documented HAMA responses have been seen, although a probable anti-idiotypic response has been seen in a single patient. By comparison the use of A5B7 has been associated with documented HAMA responses in 6 out of 7 therapies. However, to entirely attribute this apparently reduced immunogenicity of ^{131}I -CHT-25 to better antibody design is not possible since heavily pre-treated lymphoma patients are known to have a high degree of anergy even to murine antibodies (187). By contrast, more immunocompetent patients with solid tumours have been shown to be at significant risk of HAMA formation even when exposed to chimeric antibody with theoretically reduced immunogenicity (66). A direct comparison of the immunogenicity of ^{131}I -CHT-25 with ^{131}I -RFT5, its murine parent, would be a better way to assess any reduction in immunogenicity associated with its increased humanisation.

HAMA formation, in addition to being a contraindication to repeat therapy, also affects the biodistribution of antibody. Antigenicity is thought to be responsible for the short serum half-lives of therapeutically administered murine antibodies which are generally in the range of 18–48 hours in man. The reduced immunogenicity of chimeric antibodies is reported to be associated with increased stability in the circulation and a longer circulating half-life (388). In this comparison of the biodistribution of ^{131}I -A5B7 with ^{131}I -CHT-25,

considerable differences in biodistribution are evident. In whole body, blood, and all visceral organs, ^{131}I -CHT-25 has cleared more slowly than ^{131}I -A5B7. For example, in their respective study populations, whole body clearance half-lives were 26.9 and 66.9 hours for ^{131}I -A5B7 and ^{131}I -CHT-25 respectively. These differences in biodistribution mean that residence times in normal tissue are significantly longer for ^{131}I -CHT-25 than ^{131}I -A5B7 for any given activity and, as a result, lead to both higher absorbed dose and dose rates. For example, for each study population, MIRD predicts that for an administered activity of 740 MBq/m² the total marrow absorbed dose for patients receiving ^{131}I -A5B7 or ^{131}I -CHT-25 would be 0.55 and 1.48 Gy respectively. However since the dose limiting toxicity in non-myeloablative RIT is the bone marrow, the maximal safely administered activity will be determined by the marrow absorbed dose. It is useful therefore to compare the biodistributions of ^{131}I -CHT-25 and ^{131}I -A5B7 when administering an activity which would deliver an identical dose to the marrow for each antibody. In this comparison it can be seen that the chimeric antibody delivers radiation for a longer time, but also at a lower dose rate in both normal tissues and tumour.

Concerns about increased normal tissue toxicity when using chimeric and humanised antibodies with prolonged circulating half-lives, has been an important factor in the development of the two commercially available CD20 targeting antibodies. As a consequence both ^{131}I Tositumomab (*Bexxar*) and ^{90}Y Ibritumomab-tiuxetan (*Zevalin*) are based upon murine antibodies, despite the higher risk of HAMA formation. However the justification for using murine antibodies rather than their chimeric or fully humanised counterparts should

ultimately depend upon the therapeutic ratio. In this analysis, increased toxicity following the use of ^{131}I -CHT-25 as a result of its prolonged circulating half-life, could be avoided by administering a lower activity. It may be that the slower delivery of radiation to normal tissues by ^{131}I -CHT-25 as a result might be expected to reduce late normal tissue toxicity, although sparing of acute responding bone marrow elements would not be expected. However these reduced dose rates in tumour might be anticipated to be detrimental to tumour kill by allowing for greater repair of sub-lethal radiation damage.

A prediction of the possible consequences of chimerisation upon the therapeutic ratio is available from the literature using the large body of clinical data accumulated using murine CD20 antibodies and a smaller, but accumulating body of clinical data, from RIT using radiolabelled Rituximab (389). In order to perform a direct 'intra-patient' comparison of the therapeutic ratio achievable using radiolabelled Rituximab in comparison with its radiolabelled parent antibody, Ibritumomab, would be most informative. However to date Ibritumomab has only been radiolabelled with ^{111}In and ^{90}Y . However Tositumomab, the other extensively investigated murine anti-CD20 antibody has been almost exclusively labelled with ^{131}I in clinical trials. It could be argued therefore that since both Tositumomab and Ibritumomab target the same antigen, a comparison between radioiodinated Rituximab and Tositumomab could reasonably address this question (389). A comparison of published dosimetric data using ^{131}I -Rituximab with ^{131}I -Tositumomab suggests that the whole body dose is 2.7 times higher for the former but may not be associated with any increase in tumour dose, leading to reduced tumour to normal tissue

ratios (165;390). However, given the difficulty in estimating tumour absorbed doses, and the complex radiobiological effects of fractionation, it is perhaps more useful to compare therapeutic outcomes in conjunction with radiation dosimetry. With regard to this, in two recent studies both delivering a whole body dose of 75cGy, no significant differences in either response rates or toxicity were seen using either ^{131}I -Rituximab (391) or ^{131}I -Tositumomab (163;171).

In this current study the tumour absorbed doses achieved by ^{131}I -CHT-25 and ^{131}I -A5B7 antibodies appear modest. Even for radiosensitive lymphomas, the likelihood of seeing any tumour responses for the absorbed doses achieved here seems unlikely. However the accuracy of tumour absorbed dose estimates by MIRD is likely to be suboptimal. Firstly MIRD can only estimate the tumour self absorbed dose, secondly the currently available method in Olinda assumes that all tumours have a spherical shape. In addition the determination of tumour radioactivity by ROI definition of SPECT images presents further problems given its poor spatial resolution. This latter problem could be overcome by the use of correlative multi-modality imaging such as CT-SPECT, which allows anatomical information to be gathered simultaneously with activity distribution from SPECT. However continuing to use MIRD is still far from ideal because the technique assumes homogeneous activity distribution within tumour which may underestimate the effectiveness of the tumour radiation dose. Pre-clinical studies have demonstrated that selective antibody uptake in the well perfused areas may lead to enhanced biological effectiveness by specifically targeting cells in the actively growing areas of tumour (29).

The correlation between haematological toxicity and marrow absorbed dose, predicted by MIRD, has been analysed using regression analysis. This has revealed significant correlations between absorbed dose and platelet, total white cell count and neutrophil toxicities. However the ability of *individual* biodistribution data such as whole body AUC or blood AUC to predict toxicity is more reliable. One explanation for the poor ability of MIRD dosimetry to predict marrow toxicity compared to simpler but individualised biodistribution data is the wide variability in pharmacokinetics observed in both the ^{131}I -A5B7 and ^{131}I -CHT-25 study populations. In addition the biodistribution data available from patients undergoing repeated therapies suggests that intra-patient variability upon repeated exposure to antibody appears less marked than that seen for the study populations as a whole and would support the use of tracer studies to predict patient pharmacokinetics for later therapy. The use of tracer doses to define individual patient doses is already used in therapy with ^{131}I Tositumomab (*Bexaar*) (161). However the algorithms used to deliver individualised treatment in the ^{131}I Tositumomab regime are still ultimately a simplification, relying upon the whole body contribution to marrow absorbed dose. The application of individual patient dosimetry techniques, which would account for all sources of radiation, would give a truer estimate of absorbed dose. The determination of tumour and normal tissue absorbed doses using these techniques would be more accurate and allow for increasingly robust dose response relationships to be constructed.

In order to produce these more accurate dose response curves more advanced dosimetric models are required that are able to utilise patient specific geometry and also take account of the heterogeneous activity distributions that SPECT imaging provides. Sophisticated methods are currently being developed which couple data obtained from three dimensional sets of images representing the radionuclide distribution (SPECT/PET) and anatomical information obtained from CT or MRI (392-397). Monte-Carlo based approaches enable patient specific information in terms of anatomy and tissue inhomogeneity to be incorporated directly. However statistically acceptable answers require extended computational time since simulations require that numerous pseudo particles are generated, representing each radioactive decay. Moreover the decay histories of each particle must be followed and as a consequence the application of such programs is generally only feasible in institutions with access to significant computational facilities. Furthermore the surface of the organs described in Monte-Carlo code is usually required to be in analytic form such as spheres, cylinders, planes etc. whereas patients' organs have an arbitrary geometric shape. In order to address this problem another approach that has been taken is to use a beta point source function which allows for true patient geometry to be used. However such methods introduce another source of error in that they generally assume a homogeneous medium without tissue interfaces. Also, like Monte-Carlo techniques, considerable computational time is required; in one study to estimate dose contours for a single organ, an overnight calculation was required (398).

3.8 Conclusions

In this study, the information added to a simple activity escalation study by pharmacokinetics is considerable. The chimeric antibody ^{131}I -CHT-25 has a markedly different biodistribution pattern compared to ^{131}I -A5B7. This means that for an equivalent dose to bone marrow, radiation is delivered at a lower dose rate but for a longer time course in both normal tissues and tumour. The pharmacokinetic variability seen between patients using these radio-iodinated antibodies supports adoption of tracer dose studies to determine individual patient pharmacokinetics which could then be used to individualise patient dosing in subsequent therapy. The latter could be achieved using whole body clearance time as a surrogate for marrow absorbed dose and this approach is already used with ^{131}I -Tositumomab and ^{131}I -Rituximab. However, if tracer studies were combined with individual patient dosimetry even more accurate patient dosing could be achieved since all sources of radiation exposure in tissues, not just the whole body component, would be accounted for.

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My contribution to this trial has been patient recruitment, treatment, follow-up and overall care of patients treated in the study. I performed the analysis of the SPECT data and also performed the quantitative PET data analysis. I have been responsible for the collection and integration of the data for this trial and its interpretation in an attempt to improve understanding of RIT using ^{131}I CHT-25.

Data obtained from analysis performed by someone other than myself is indicated in the table below. This trial is supported by Cancer Research UK

Contributors

Professor Richard Begent¹

Dr Peter Amlot²

Dr Surinder Sharma¹

Dr Andrew Hilson³ and Dr John
Buscombe³

Dr Alan Green¹, Sandra Baig,
Sweta Parker¹

Claire Cruikshank⁴, Julie Wren⁴
drug development office

Nuclear Medicine department
Royal Free Hospital

Contribution

Principal investigator. Recruitment,
clinical care and management of patients.

Co-investigator, Recruitment, clinical
care and management of patients. FACS
analysis

Immunogenicity measurements-HAMA
by ELISA

Qualitative FDG-PET and SPECT
interpretation

Gamma camera scanning of patients and
reconstruction of data, gamma counter
calibration and analysis of samples.

Data monitoring and data entry at CRUK

Radiolabelling and external
whole body dose rate measurements

¹Cancer Research UK Targeting and Imaging Group, Department of Oncology,
Royal Free and University College Medical School, University College, London
NW3 2PF

²Department of Immunology, Royal Free and University College Medical
School, University College, London NW3 2PF

³Department of Nuclear Medicine, Royal Free and University College Medical
School, University College, London NW3 2PF

⁴Drug development office, Cancer Research UK, London WC2A 3PX

4 **An Investigation of the effects of dose fractionation upon**
tumour growth and early and late haematological toxicity in nude
mice bearing LS174T human colorectal xenografts treated with
¹³¹I labelled monoclonal antibody A5B7

4.1 **Introduction**

Colorectal cancer is the second leading cause of cancer death in the UK, being responsible for 11% of all cancer deaths and resulting in nearly 17 000 deaths per annum in England and Wales (399). The incidence of colorectal cancer is 48/ 100,000 per year in the UK (399). The current 5-year survival for patients with all stages of colorectal cancer is 40% whereas the median survival for patients with metastatic disease is only 11 months (400).

Surgery remains the only established curative treatment for colon cancer, with chemotherapy used as adjuvant treatment. 5 Fluorouracil (5FU), an anti-metabolite with predominantly S-phase activity, has historically been the mainstay of chemotherapy for colorectal cancer. In the adjuvant setting a meta-analysis of 39 randomised clinical trials in resected colorectal cancer has revealed an overall survival benefit of 5% for colon cancer and 9% for rectal cancer by the addition of adjuvant chemotherapy (401). Moreover the IMPACT 2 study has demonstrated no increase in overall survival or event-free survival for patients with Dukes' B tumours, and as a result Fluorouracil based chemotherapy is not routinely recommended as a standard adjuvant treatment for patients with Dukes' B colon cancer (402)

Conventional chemotherapy is unable to cure patients with metastatic disease although its use is associated with response rates of approximately 20-30% with infusional regimens achieving a slightly longer overall survival time (403;404). The use of systemic 5FU-based chemotherapy is associated with significant patient morbidity including haematological toxicity, a plantar-palmer syndrome and diarrhoea, nausea and vomiting, or mouth ulcers in approximately 15% of patients (404).

Recently two new chemotherapeutics have been shown to have efficacy in advanced colorectal cancer. These are Irinotecan (CPT11), a topoisomerase I inhibitor, and Oxaliplatin, a synthetically-derived platinum. Single agent Irinotecan has an objective response rate of 13% as second line treatment for metastatic disease, with another 40-50% of patients achieving stable disease (405). An increased median survival of approximately 2 months has been shown in two randomised controlled trials comparing single agent Irinotecan administered after the failure of 5FU compared to best supportive care or an infusional 5FU regimen (406;407). As a first line treatment for metastatic colorectal cancer, Irinotecan in combination with 5FU has a response rate of 39-41% compared to 21-23% with 5FU alone, with a statistically significant improvement in time to progression and overall survival (408;409). The side-effects associated with Irinotecan are diarrhoea, myelosuppression (neutropenia), acute cholinergic syndrome, nausea and vomiting. Grade 3 or 4 diarrhoea has been reported in 20-40% of patients with single agent Irinotecan or Irinotecan in combination with 5FU (410;411). Grade 3 or 4 neutropenia has been reported in up to 54% of patients (412).

Single agent Oxaliplatin used as first line chemotherapy for metastatic colorectal carcinoma has a response rate of approximately 22%, which is comparable with single agent 5FU (413). Significant synergy has been shown in vitro between Oxaliplatin and Fluorouracil, so most clinical trials have utilised this combination. In first-line therapy for metastatic disease the combination of Oxaliplatin with 5FU has resulted in a response rate of approximately 50% (414;415). The toxicities seen include neutropenia, diarrhoea, peripheral neuropathy, nausea and vomiting (416;417). There were improvements seen in time to progression, but overall survival was not significantly enhanced by the addition of Oxaliplatin to a 5FU regimen (418;419).

Therefore, despite the introduction of both Irinotecan and Oxaliplatin into the management of advanced colorectal cancer and the increased response rates, treatment still remains essentially palliative and long-term cures have not been achieved. Improvements in survival have, in some trials, been attained, but these improvements have been in terms of months, rather than years and therapy is associated with significant toxicities for patients.

As an alternative approach in colorectal cancer, RIT using CEA as the target antigen has been extensively investigated. However whereas RIT has achieved considerable success in the treatment of the lymphomas and leukaemias (163;169;420;421), success in the treatment of the common epithelial tumours has been less impressive (66;232;236). In a recent review of RIT of colorectal cancer looking at a large number of phase I/II clinical studies in which a variety

of antibodies and radionuclides targeting a number of tumour associated antigens have been used, only modest activity has been seen (422). The reasons for the failure of RIT are multifactorial, but include the inability of RIT to deliver an adequate dose to tumour, and as a result RIT of colorectal cancer remains investigative.

In RIT the maximum administered activity, and hence the maximum tumour dose, is limited by haematological toxicity. In order to address this problem one technique that has been adopted is the use of stem cell support, which has allowed for significant dose escalation in the RIT of both lymphomas (169) and solid tumours (423;424). However many patients are not suitable for such approaches as they are associated with an appreciable morbidity and mortality.

Fractionation of EBRT, that is dividing the total therapy dose into a number of smaller doses, is an established technique to reduce late normal tissue toxicity. This allows for significant increases in the total radiation dose that can be delivered (425). Fractionation has therefore also been proposed as a method to reduce normal tissue toxicity and allow for dose escalation of RIT so improving its therapeutic ratio.

Moreover a number of preclinical studies have suggested that fractionation of RIT may lead to an enhancement of therapeutic effect even in the absence of dose escalation. In the study by Schlom et al using the whole monoclonal antibody B72.3 in a murine xenograft model, and where the same total administered activity was divided into 2 or 3 fractions, enhanced anti-tumour effects were seen as a result of fractionation (326). Another study looking at

fractionation of the equivalent administered activity using the single chain Fv CC49 similarly revealed a significant advantage to tumour control in the fractionated group compared to the single fraction arm (325). Buchsbaum et al using the CC49 whole antibody also observed a significant reduction in tumour growth when the same total activity was given as two fractions rather than a single fraction, but only when given three days apart. Increasing the inter-fraction interval to 7 days resulted in a marked loss of efficacy with no tumour regressions seen (323). A more recent study looking at fractionated intra-peritoneal low dose RIT in a murine gastric cancer tumour model using a ^{213}Bi conjugated monoclonal antibody has similarly demonstrated a therapeutic advantage to the administration of double injections of antibody at a seven day interval in comparison to a single injection of twice the activity (328).

The purpose of this study therefore was to investigate the therapeutic efficacy and toxicity of ^{131}I labelled A5B7, a monoclonal antibody binding to CEA, as either a single treatment or as a fractionated regimen in nude mice bearing CEA expressing LS174T xenografts. A previous study using ^{131}I labelled A5B7 fragments did not demonstrate a therapeutic advantage to fractionation of a given activity into three separate doses (246). However in this study only a single fractionation regime was investigated and no direct assessment of haematological toxicity was made. In this study therefore a range of fractionation regimes has been investigated and in addition a more detailed assessment has been made of haematological toxicity. This has comprised measurement of both acute changes in mature blood indices and also an assessment of haematopoietic stem cell function following therapy

4.2 Materials and Methods

4.2.1 Antibody and Radiolabeling

The monoclonal antibody A5B7 used in this study has been described previously (426) and has been used pre-clinically and clinically for the RIT of CEA expressing tumours (232;246;320;427-429). The antibody was radiolabelled on Day 1 using the chloramine-T method (430) and sterilised by passage through a 0.22 μm acrodisc filter.

4.2.2 Animal Model

The human colorectal adenocarcinoma cell line LS174T is a moderately to poorly differentiated CEA producing adenocarcinoma with small glandular acini (431) and was used to develop a tumour xenograft in the flanks of female nude (*nu/nu*) mice by subcutaneous implantation of small tumour pieces ($\sim 1\text{mm}^3$). At the initiation of the experiments, all mice were aged 2-3 months and weighed 23-27g.

4.2.3 Biodistribution Studies

Antibody biodistribution was assessed by administering 1.8 MBq of ^{131}I labelled radiolabelled antibody by tail vein injection into mice bearing LS174T xenografts (approximately 0.75cm^3). At selected time points the animals were bled and liver, kidney, lung, muscle, colon, spleen and tumour removed for comparative activity assessment using a LKB Wizard (Pharmacia) gamma counter. Results are expressed as percentage injected dose per gram of tissue and were the mean values from four mice sacrificed at each time point.

4.2.4 Therapy Studies

Experiments commenced 7-10 days following passaging when the tumours were in exponential growth and had reached 0.1-0.2 cm³ in size. Groups of six mice received either single or multiple tail vein injections of radiolabelled antibody at a range of activities to give a total of 7.4 MBq; control mice underwent tail vein injection of normal saline. Tumours were measured every 2-3 days and mice were culled by cervical dislocation when the tumour volume reached 1.5 cm³. Tumour measurements were carried out in 3 dimensions (length, width and height) and the tumour volume estimated as length x width x height/2 (432).

4.2.5 Toxicity Studies

Systemic toxicity was assessed by animal weights, with animals weighed on the day of antibody injection and regularly every 2-3 days thereafter. Acute and late haematological toxicity was assessed using full blood counting and assays of early progenitor cell function.

Acute blood toxicity

Mice underwent weekly removal of 25 µl of whole blood by retro-orbital puncture for 1 month following initiation of therapy. Following dilution in physiological media this blood was then analysed using an automated haemocytometer in order to give haemoglobin, white cell and platelet differentials.

Late blood toxicity

Upon sacrifice, marrow was taken from the mouse femurs in order to perform colony forming unit assays for granulocytes and macrophages (CFUgm). Using aseptic technique a superficial incision was made into the lower abdomen of the

mice to pierce the skin. Subsequent removal of the skin of the lower limbs and a limited dissection of soft tissue from the femur was then performed to expose the knee and hip joints. An incision was then made through the proximal femur at the hip joint and just proximal to the knee. The marrow contents were flushed from the marrow cavity using 2 mls of PBS and a 25 gauge needle. This cell suspension was then diluted in 3% acetic acid to lyse red blood cells and the mononuclear cells counted using a haemocytometer. 10^5 cells were mixed thoroughly with the growth medium (Methocult™ CFU-GM catalogue no. 03534), plated out and incubated at 37°C for 7 days in humidified 5% CO₂. At this point colonies were counted using an inverted microscope. Age matched controls were treated similarly.

4.2.6 Statistics

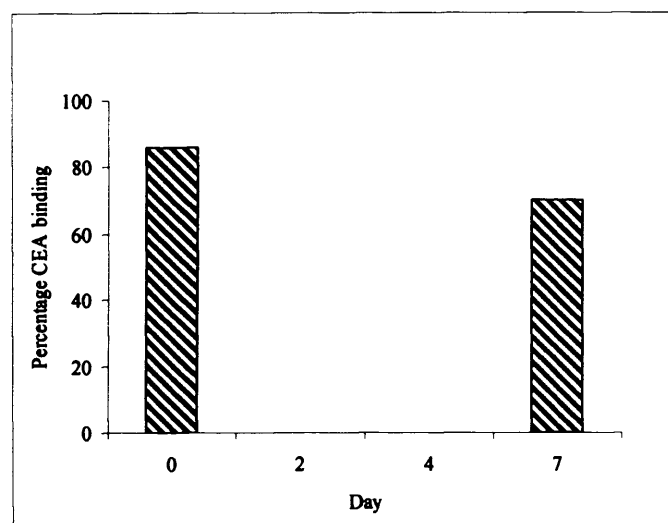
Kaplan Meier analysis was used to assess the survival of the different treatment groups with a log rank test to estimate any differences. Other inter-group analyses were performed using either a Mann Whitney-U test to compare 2 groups or a One Way ANOVA where multiple comparisons were made.

4.3. Results

4.3.1 Antibody binding

Antibody binding was assessed on Day 1 and Day 7 using a CEA binding column and revealed a modest decrease in binding efficiency over the course of the experiment, falling from 86% to 70% on Days 1 and 7 respectively and is shown in Figure 4.1.

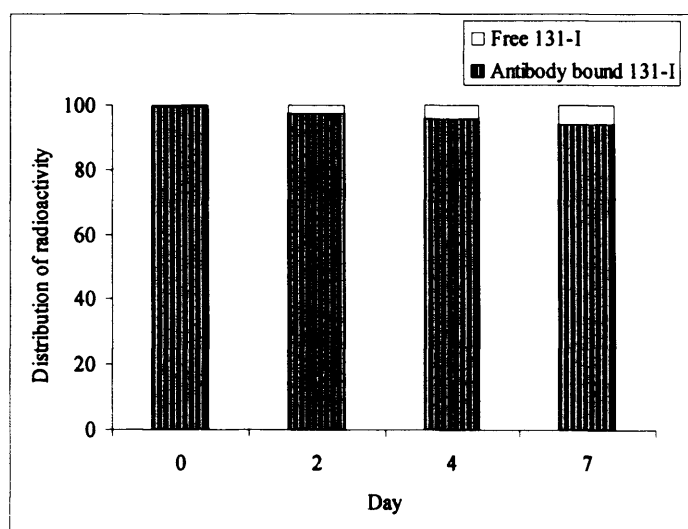
Figure 4.1: CEA binding assessed by CEA column on days 1 and 7. A modest reduction of antibody binding affinity over the course of the experiment was observed.



4.3.2 Radiopharmaceutical purity

Stability of the radioimmunoconjugate over the course of the experiment was assessed using thin-layer chromatography. This revealed a small increase in the proportion of free antibody from less than 0.5 % to 5.9% and is shown in Figure 4.2.

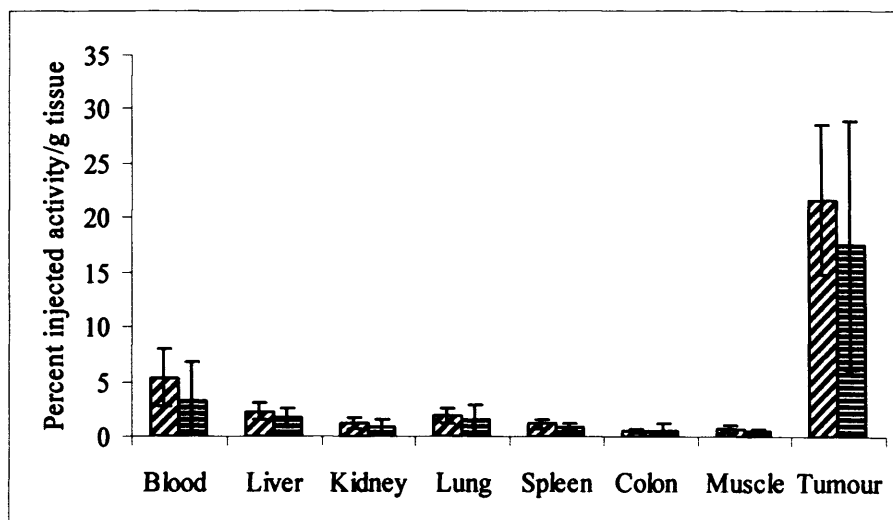
Figure 4.2: Radiopharmaceutical purity assessed using thin-layer chromatography. This revealed a minor deterioration over the course of the experiment.



4.3.3 Activity Biodistributions

Figure 4.3 shows the activity biodistribution in groups of 4 mice given 1.8 MBq of ^{131}I A5B7. Greater than 20% of injected activity was found in the tumour at 24 hours whereas blood clearance had already fallen to 5% and levels in other normal tissues were even lower. A similar picture was seen at 48 hours, with good activity retention in the tumour.

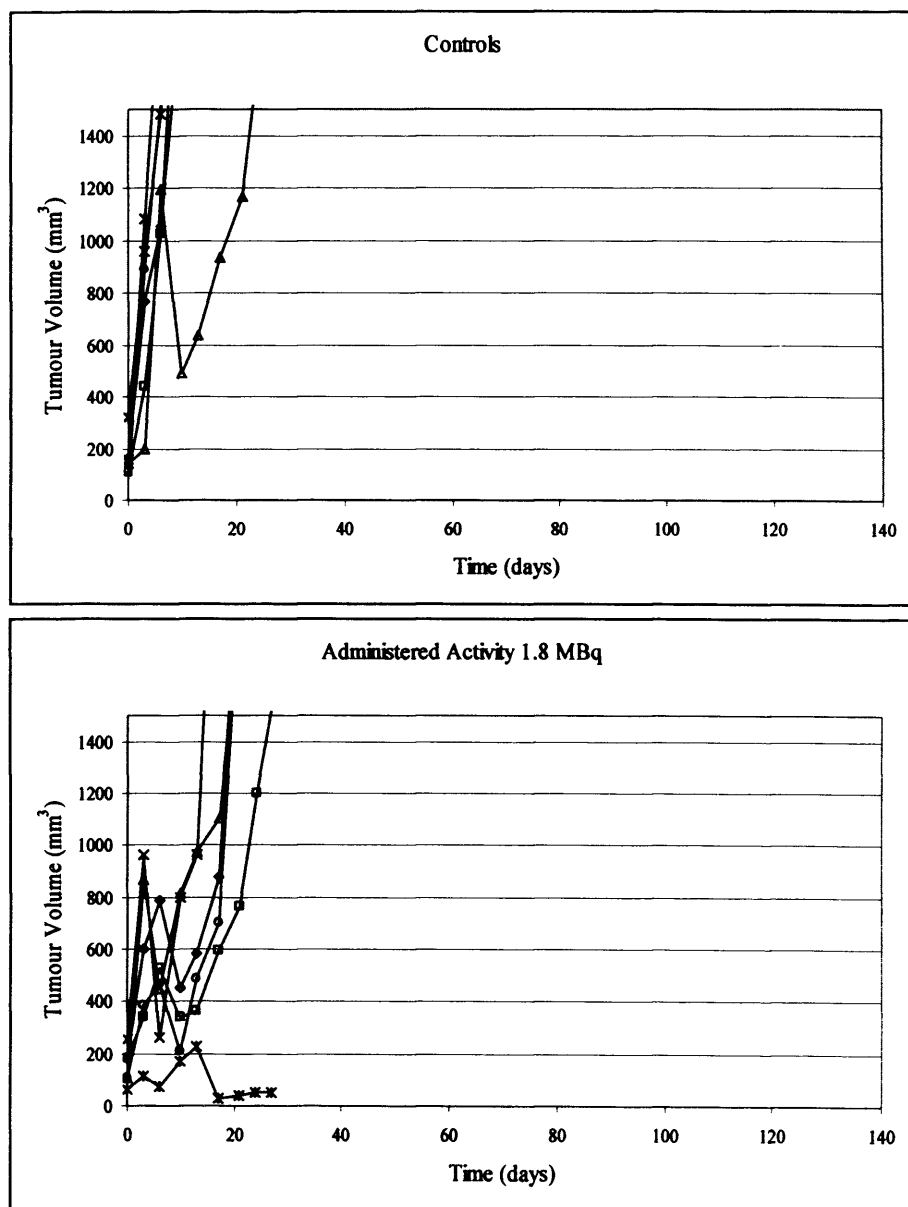
Figure 4.3: Distribution of ^{131}I labelled A5B7 at the 24 and 48 hour time points (with associated standard deviations) in the tissues of mice bearing LS174T xenografts. (n=4 at each time point).

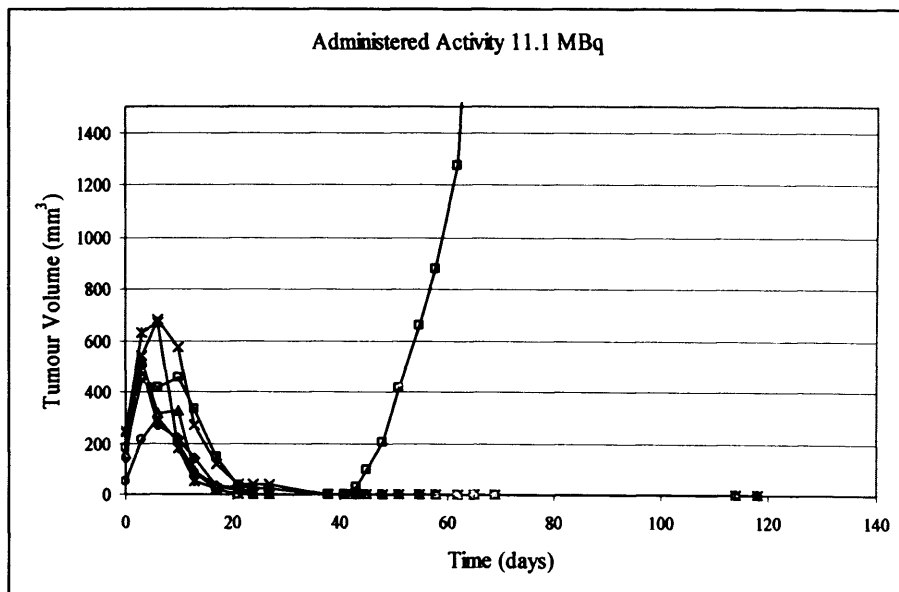
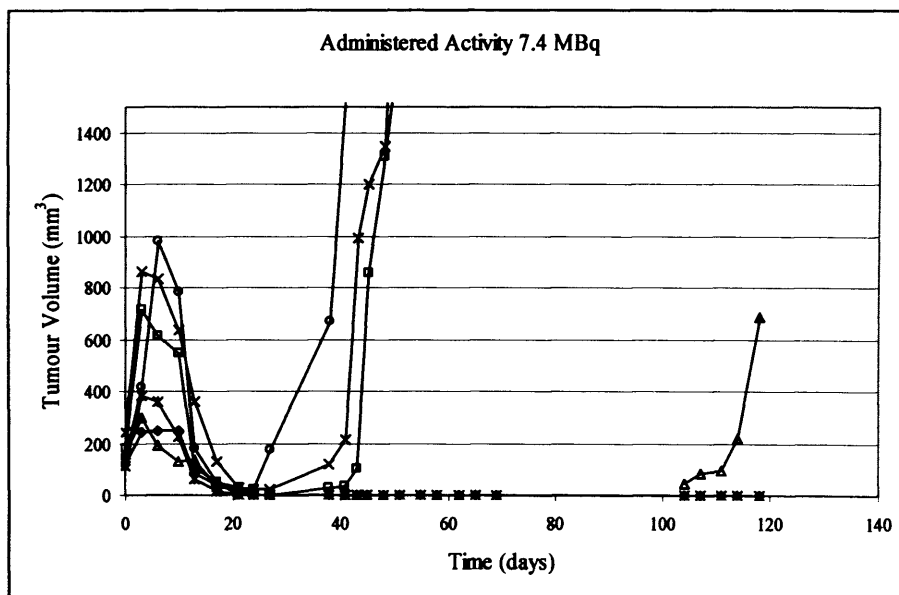
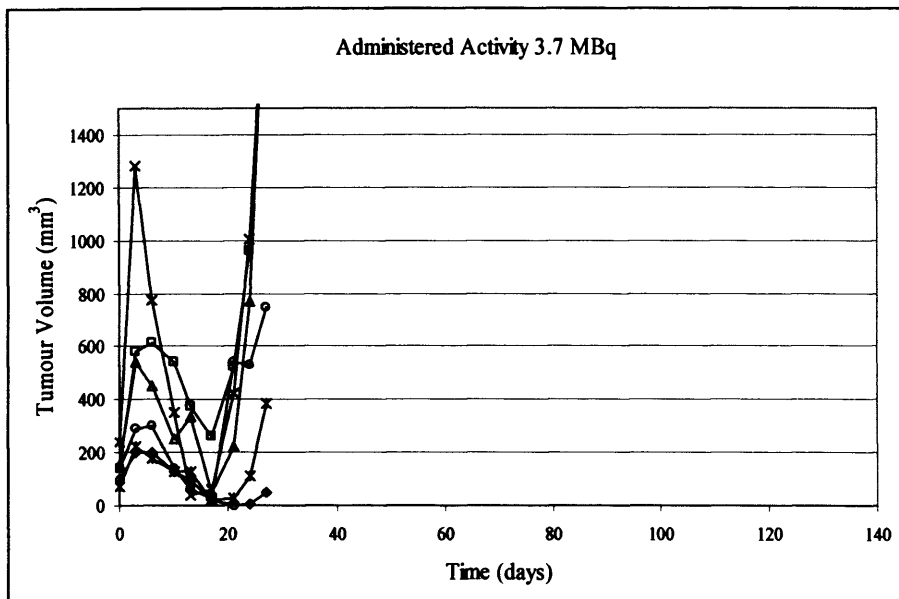


4.3.4 Therapy Studies: Preliminary single dose studies

In order to determine an appropriate fractionation schedule, a series of single therapy experiments were performed in tumour bearing mice. Administered activities ranged from 1.8 MBq to 11.1 MBq with an assessment of both tumour growth and acute haematological toxicity as detailed above. Figure 4.4 shows tumour growth for each of the single therapy studies and although growth delay was seen for each of the treated groups over control, long term control was only seen in the 7.4 MBq and 11.1 MBq groups.

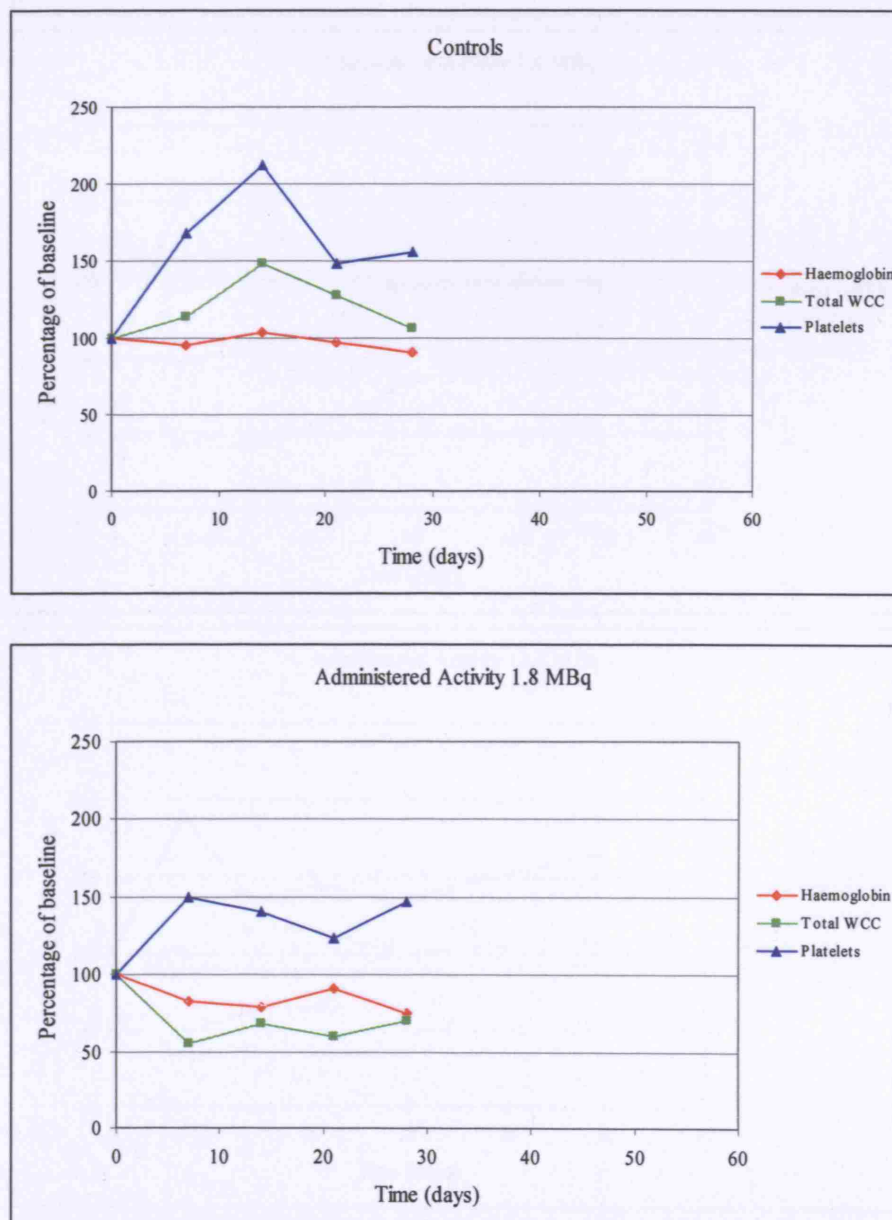
Figure 4.4: Tumour growth following single administration of a range of activities from 1.8 MBq to 11.1 MBq. (n=6 mice per group)

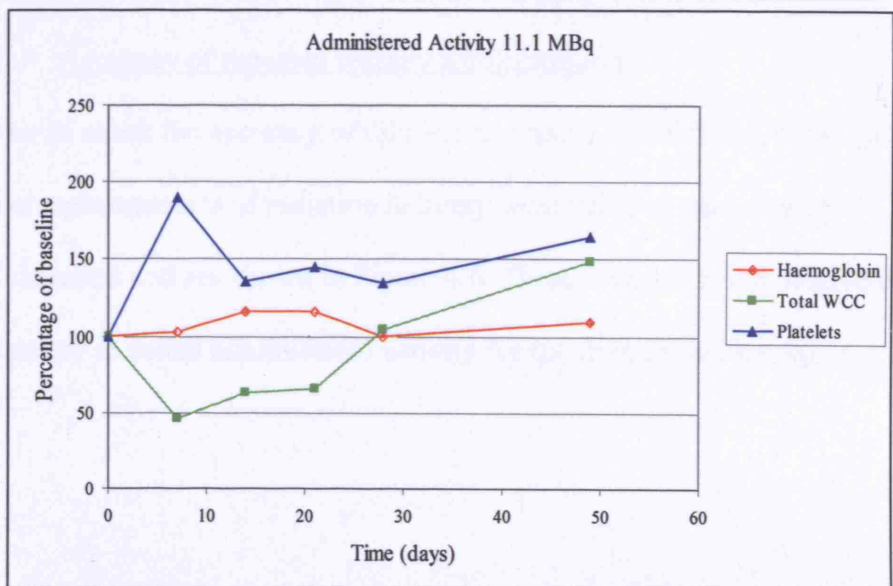
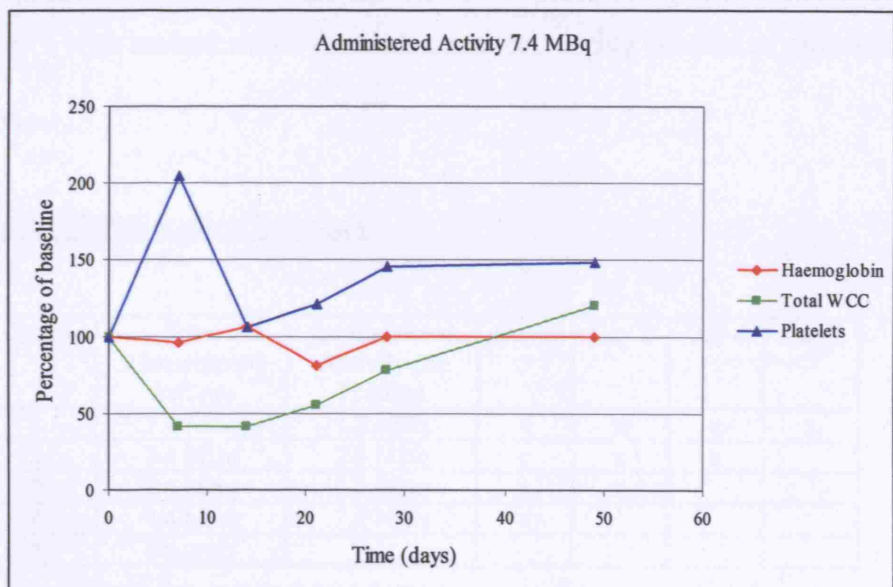
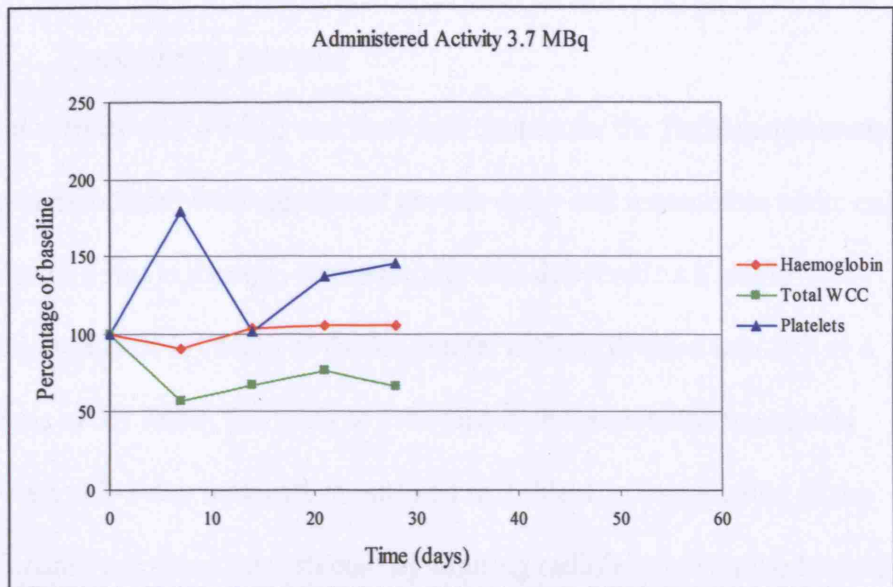




Acute haematological toxicity is shown in figure 4.5 and revealed evidence of thrombocytosis as a result of repeated phlebotomy, and acute decreases in total white cell counts which appeared to be more marked at the higher administered activities.

Figure 4.5: Acute haematological toxicity following single administration of 1.8 MBq to 11.1 MBq ^{131}I A5B7.





4.3.5 Fractionation schedule

A total activity of 7.4 MBq was therefore chosen for the fractionated study as this was associated with significant growth delay and measurable white cell toxicity as a single therapy. Radioactivity was delivered as a single administration of 7.4 MBq or the same total activity divided into 2, 3 or 4 fractions of 3.7 MBq, 2.4 MBq or 1.8 MBq with fractionated treatments delivered at 2-3 day intervals as outlined in Table 4.1. Formulation of the fractionated activities was effected by diluting radiolabelled antibody made up on Day 1 with normal saline for each treatment, taking account of radioactive decay.

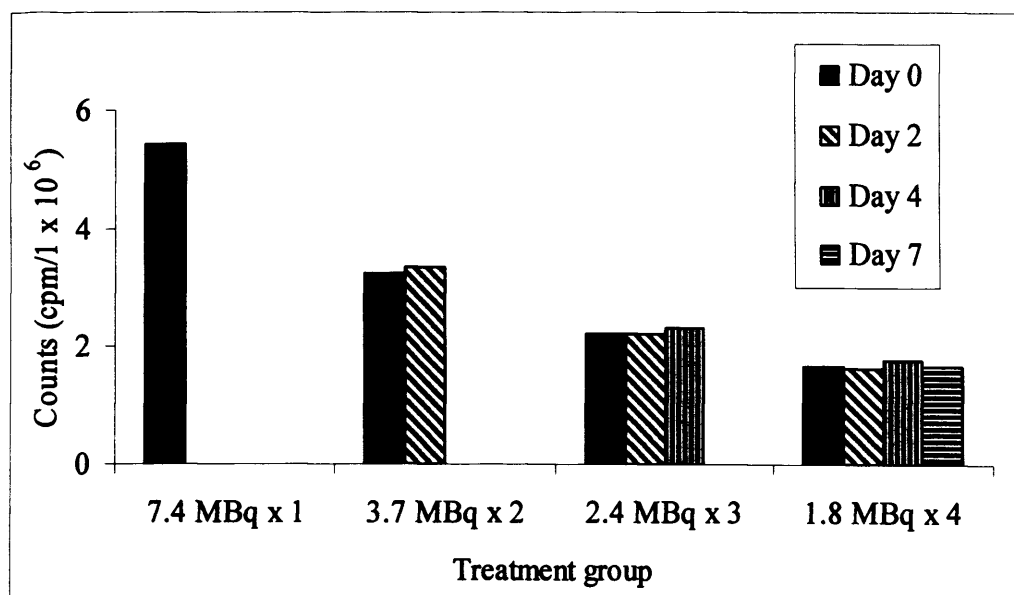
Table 4.1: Fractionation Schedule

Group	Total Administered Activity	Administered Activity per Fraction	Day 0	Day 2	Day 4	Day 7
1	7.4 MBq	1.8 MBq	x	x	x	x
2	7.4 MBq	2.4 MBq	x	x	x	
3	7.4 MBq	3.7 MBq	x	x		
4	7.4 MBq	7.4 MBq	x			
5	Controls	-				

4.3.6 Accuracy of repeated therapy administration

In order to check the accuracy of dilution and decay calculations, serial gamma counter measurements of radiation delivery were taken on each day of administration and are shown in Figure 4.6. These revealed a high degree of consistency in actual administered activity for the fractionated therapies.

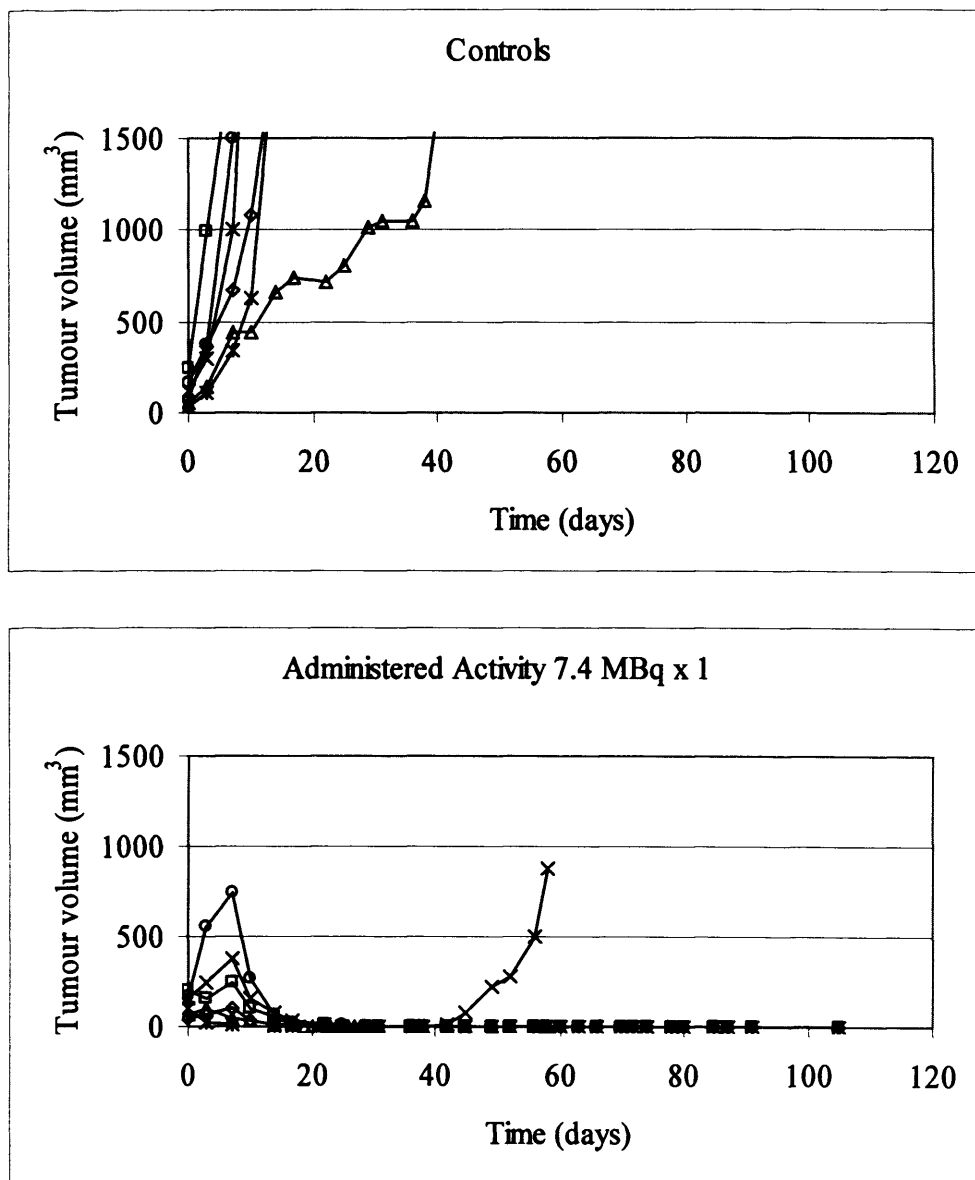
Figure 4.6: Accuracy of repeated administrations of radiolabelled antibody assessed by serial gamma counter measurements. Mean administered activity was 98.4% of baseline with a standard deviation of 3.5%.

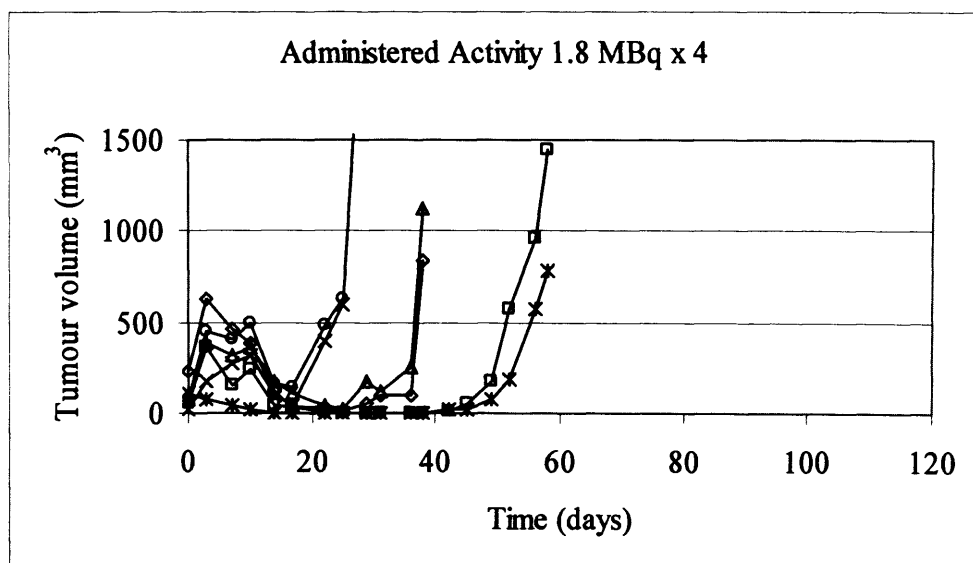
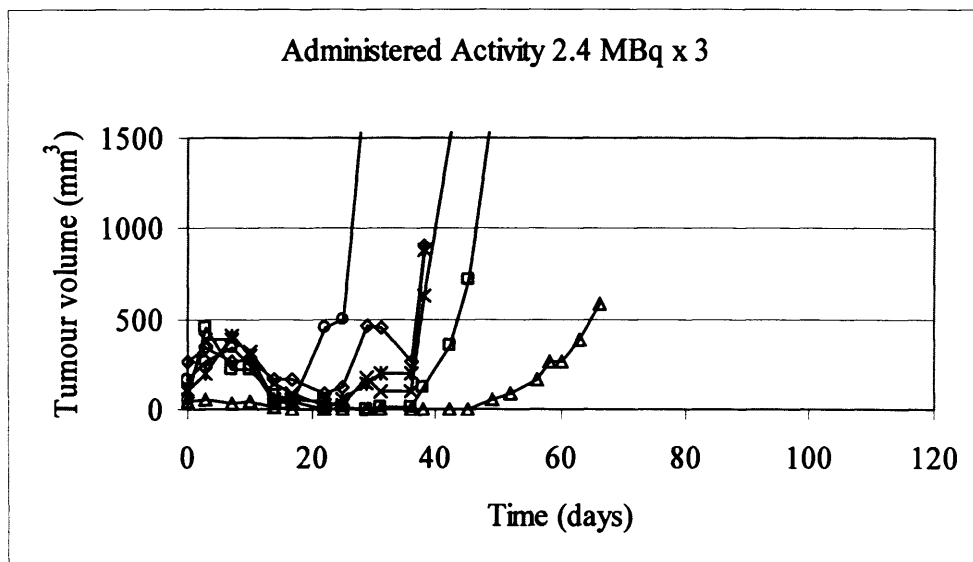
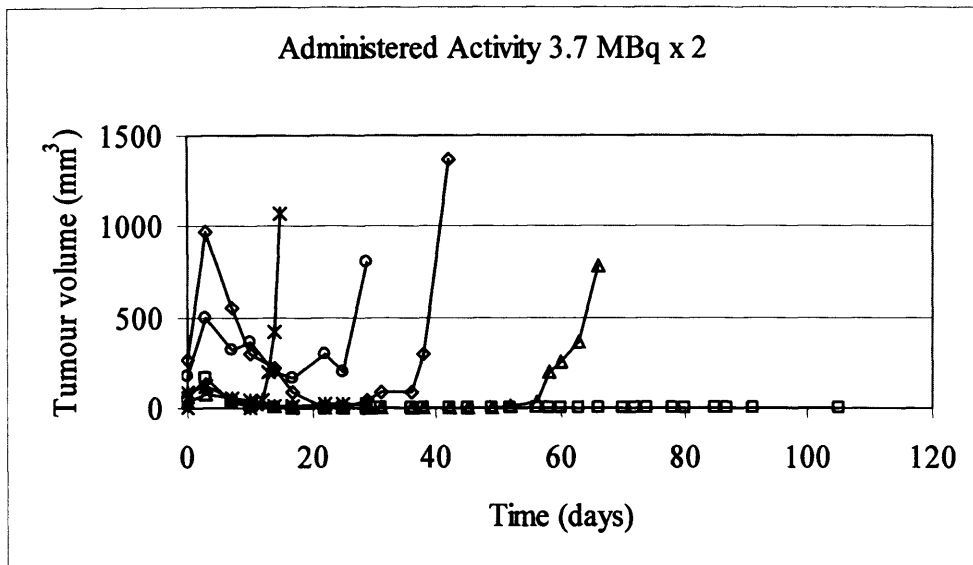


4.3.7 Tumour Growth

In the control group exponential growth continued in all but one animal. A short period of continued tumour growth was seen in all treated animals, followed by a fall in tumour size before differential re-growth (Figure 4.7). The period of tumour growth inhibition was directly related to the activity of individual therapies with cures seen in five of the mice receiving 7.4 MBq, two of the mice receiving 3.7 MBq but none of the mice in the other treatment groups during the observation period of 230 days after which the experiment was terminated.

Figure 4.7: Individual tumour growth utilising serial three dimensional measurements of tumour size to estimate tumour volume.





4.3.8 Survival

The Kaplan-Meier method (Figure 4.8) has been used to display the cumulative probability of mice surviving each of the treatment regimes and a log rank test to test the null hypothesis that there are no differences in survival between the treatment groups (Table 4.2). The analysis reveals a significant improvement in survival time between the different treatment groups with Group 1, receiving a single administration of 7.4 MBq yielding a significantly improved survival over controls and groups receiving 1.8 and 2.4 MBq but not 3.7 MBq. All the treatment groups had a significant survival advantage over the control group, however, with the 3.7 MBq group being more effective than the 2.4 MBq group, which was more effective again than the 1.8 MBq group.

Figure 4.8: Kaplan Meier Plot

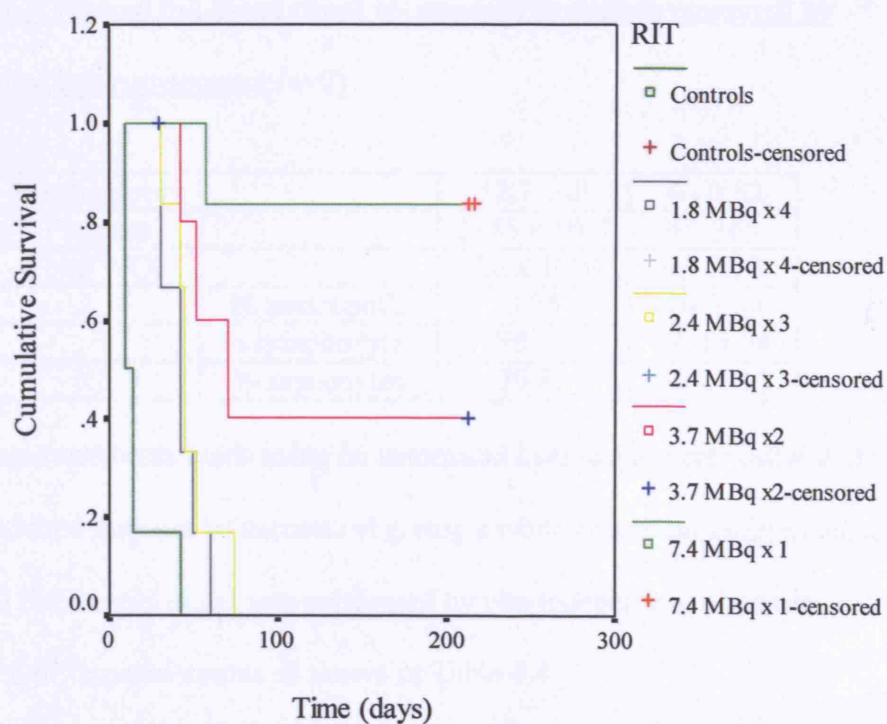


Table 4.2: Mean survival times as assessed by the log rank method.

Group	Mean Survival time (days)	Standard Error	95% Confidence interval
7.4 MBq x 1	190	24.04	143 -237
3.7 MBq x 2	118	34.92	50-187
2.4 MBq x 3	48	5.87	37-60
1.8 MBq x 4	42	4.57	34-52
Controls	17	5.13	7-26

4.3.9 Normal Blood Indices

7 anaesthetised non-tumour bearing mice were bled by cardiac puncture to determine the normal full blood count and white cell differentials and are shown in Table 4.3. In contrast to humans the total white cell count was composed primarily of lymphocytes with neutrophils contributing only 3.75% of the total cell count.

Table 4.3: Normal full blood count +/- standard deviations measured by automated haemocytometer (n=7)

Haemoglobin		12.7 g/dl	+/- 0.52
Platelets		$1145 \times 10^9/l$	+/- 365
Total WCC		$8.6 \times 10^9/l$	+/- 2.38
	% neutrophils	3.75	+/- 2.09
	% lymphocytes	55.87	+/- 15.04
	% monocytes	29.81	+/- 11.18

Since measurements made using an automated haemocytometer calibrated for human blood may not be accurate at giving a white cell count differential, a manual differential count was performed by two independent observers and revealed differential counts as shown in Table 4.4

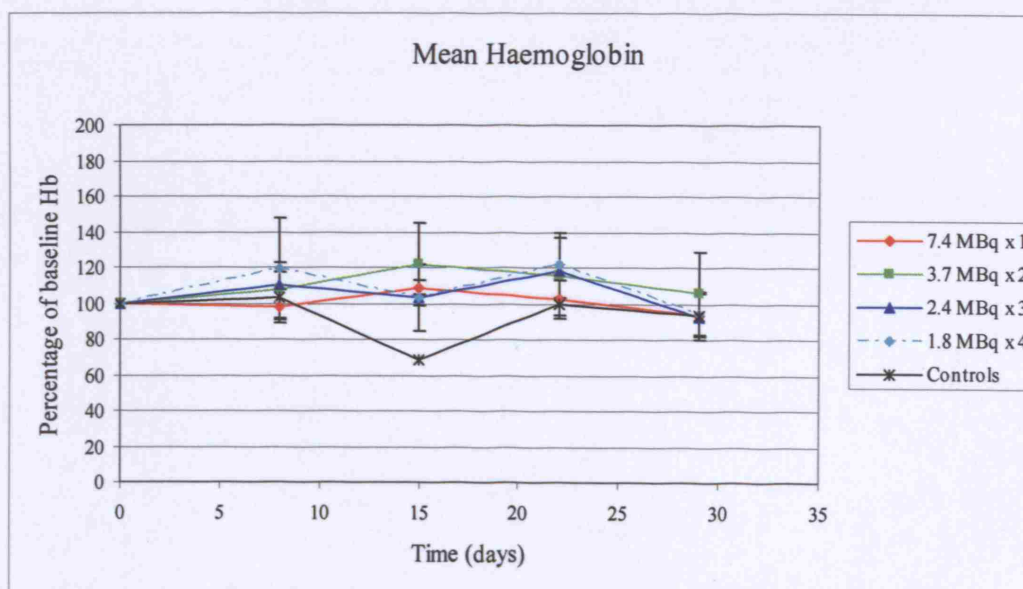
Table 4.4: Manual white cell differential counts

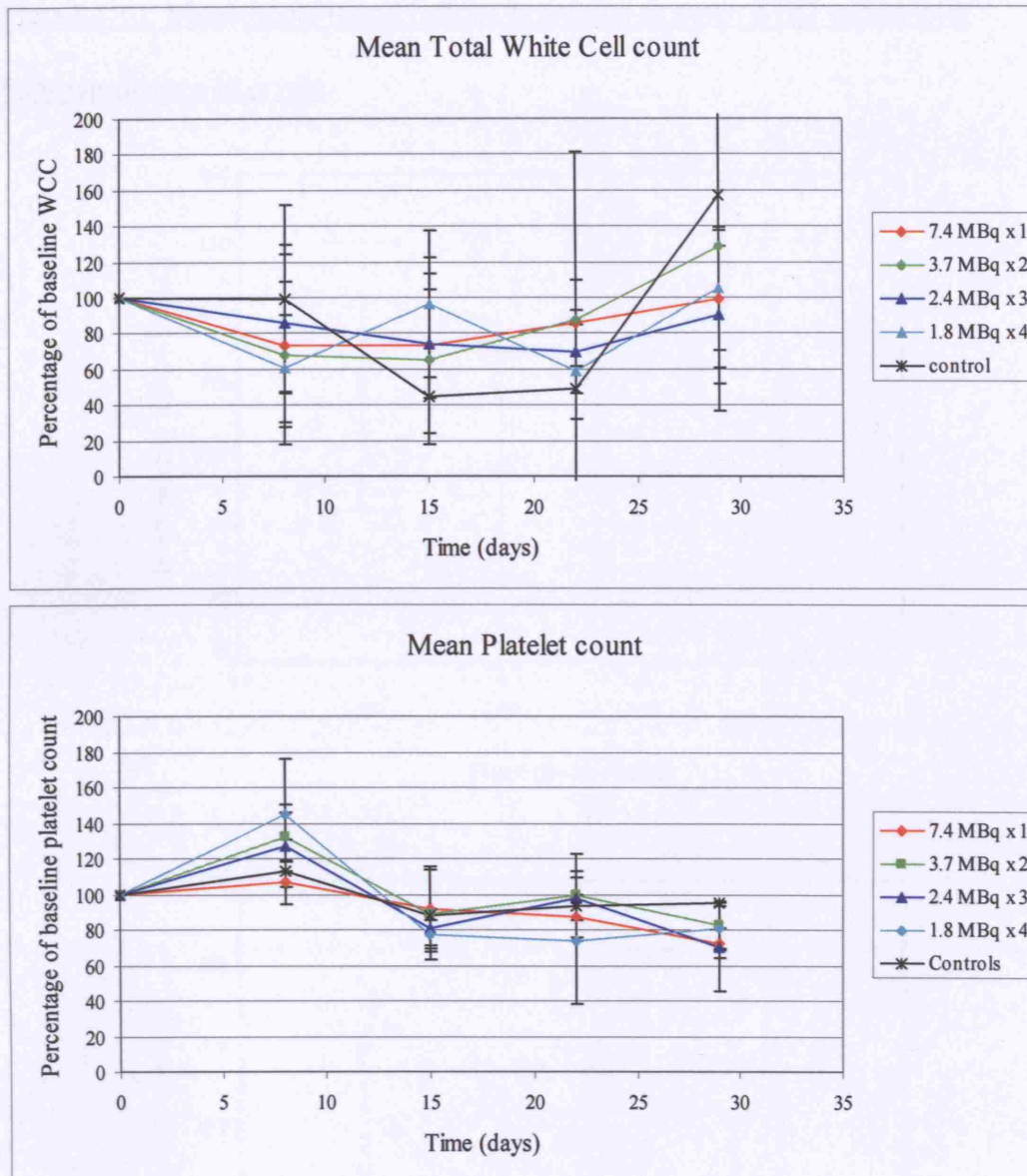
% Neutrophils +/- SD	% lymphocytes +/- SD
30.9 + 12.9	69.5 + 12.6

4.3.10 Acute blood toxicity

Due to rapid tumour progression in the control group, only a single untreated control mouse was available for comparison with the therapy arms after Day 8 and this data has therefore been excluded from the statistical analysis. Mean blood indices for each of the treatment groups are shown in Figure 4.9.

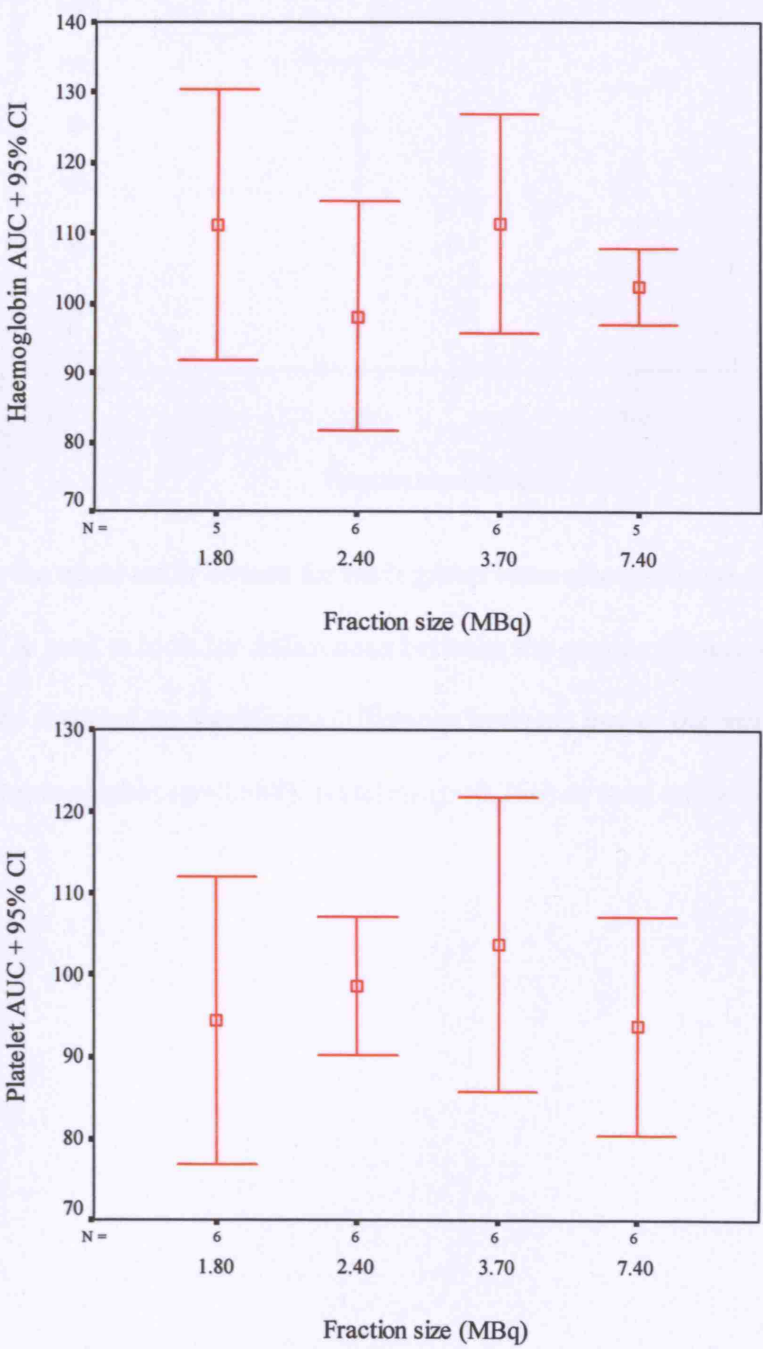
Figure 4.9: Blood indices as a percentage of baseline following therapy with associated standard deviations.

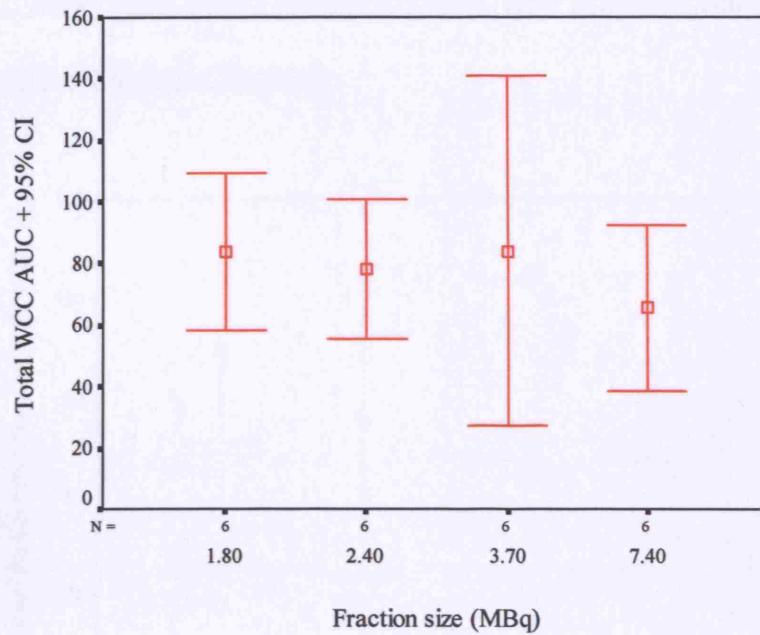




In order to look for any differences in the blood counts as a result of therapy, the mean area under the curve was calculated for each of the therapy groups and a one way ANOVA performed to look for differences between them (Figure 4.10). No significant differences between the mean AUC was found for haemoglobin ($p=0.302$), platelets ($p=0.598$) or total WCC ($p=0.763$).

Figure 4.10: Mean Acute blood indices expressed as mean AUC values with 95% confidence intervals

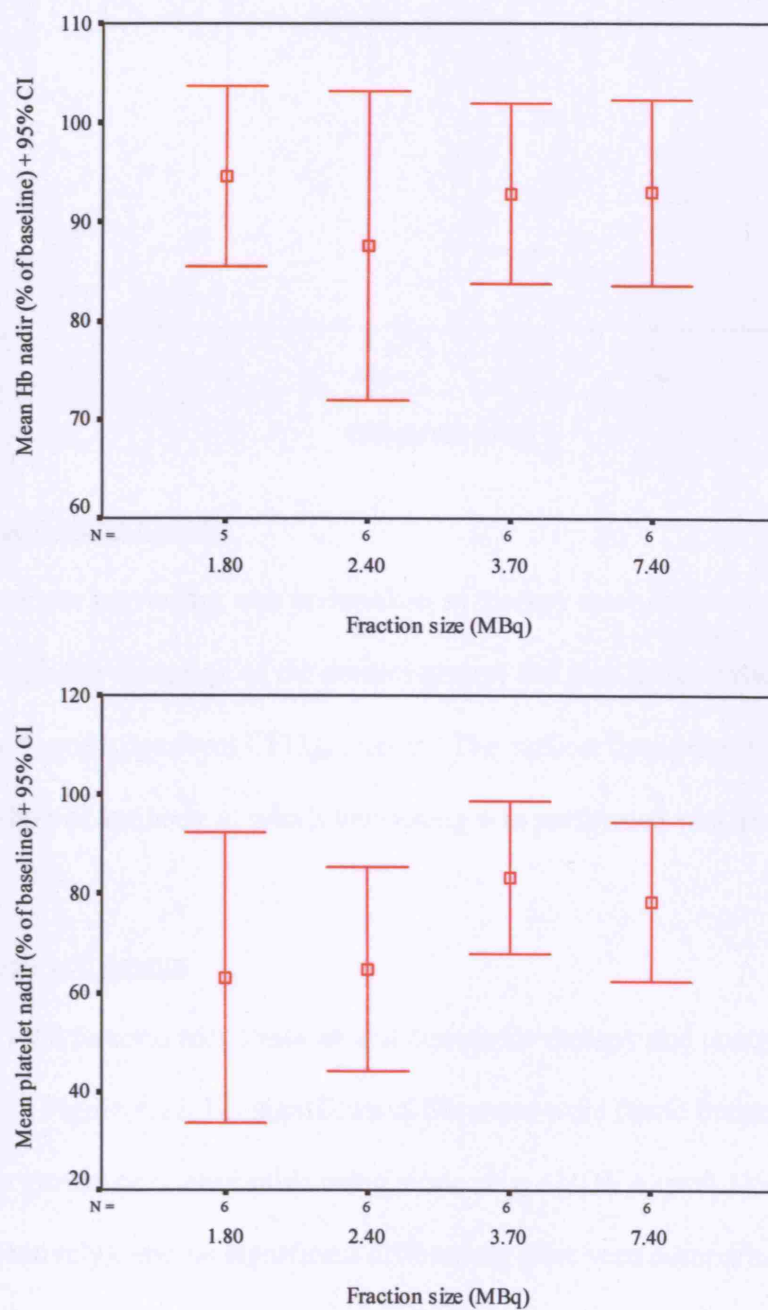


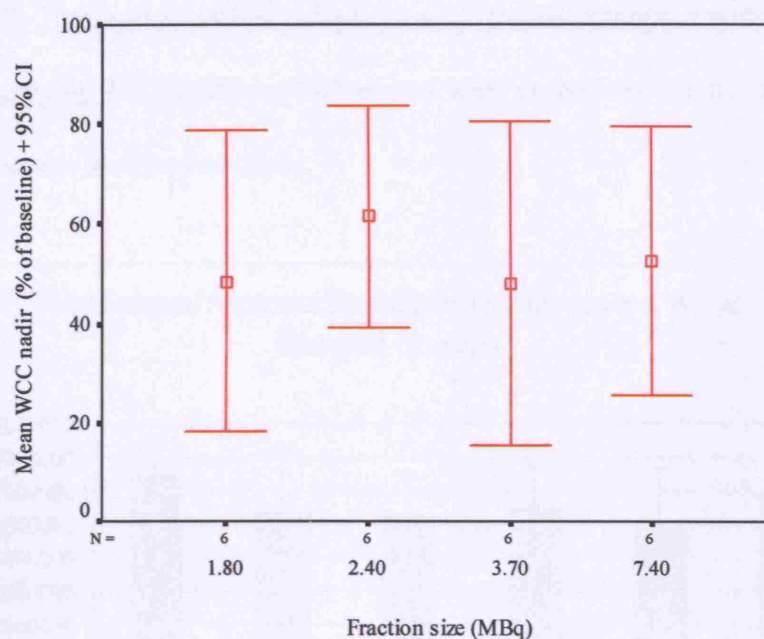


In addition, the acute nadir counts for each group were also analysed and a one way ANOVA used to look for differences between the groups (Figure 4.11).

This analysis revealed no significant difference between any of the treatment groups for haemoglobin ($p=0.688$), platelets ($p=0.256$) or total white cell count ($p=0.505$).

Figure 4.11: Mean nadir counts for haemoglobin, total white cell count and platelets + 95% confidence intervals





4.3.11 Late blood toxicity

Femoral marrow harvesting was undertaken in therapy mice at the time of sacrifice (with the exception of the control group) and also in age matched controls and used to perform CFU_{gm} assays. The earliest time point following administration of antibody at which harvesting was performed was 38 days.

Mononuclear cell counts

Estimated total femoral mononuclear cell counts for therapy and control mice are shown in Figure 4.12. No significant differences were found between any of the therapy groups or control mice using a one way ANOVA ($p=0.139$ and 0.872 respectively), and no significant differences were seen comparing the treated mice to their age matched controls for either the $3.7 \text{ MBq} \times 2$, $2.4 \text{ MBq} \times 3$ or $1.8 \text{ MBq} \times 4$ groups (Figure 4.13), using a Mann-Whitney U test ($p=0.439$, 1.0 , and 0.394 respectively).

Figure 4.12: Total mononuclear cells in treated groups compared with age matched controls. No significant differences were noted between therapy animals and age matched controls.

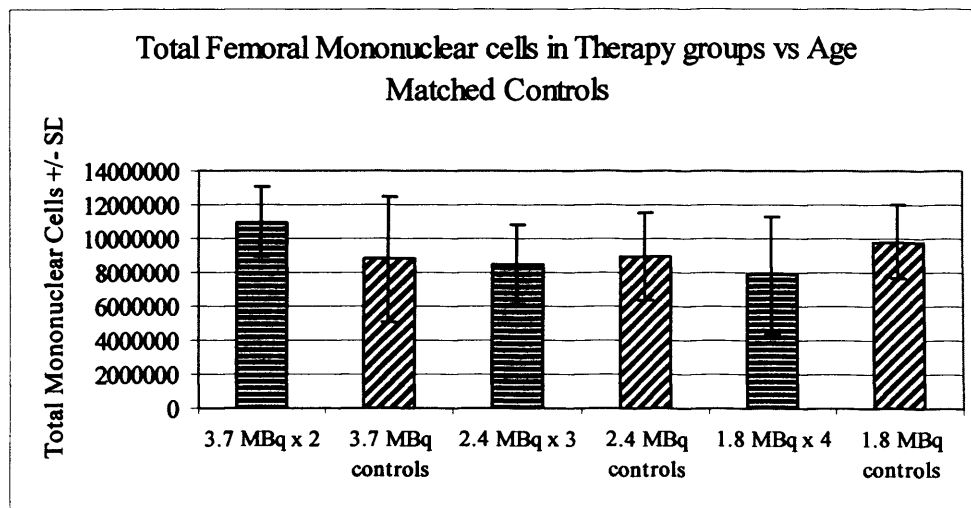
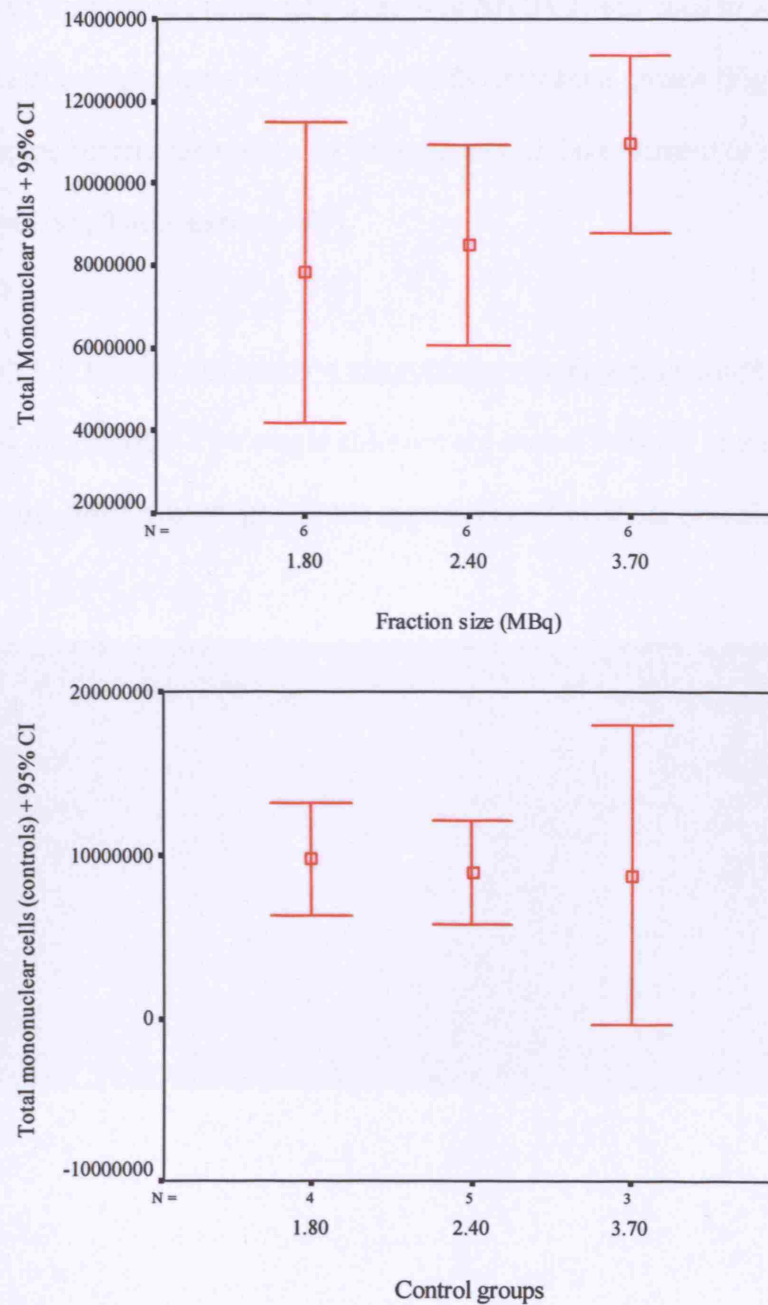


Figure 4.13: Total mononuclear cell counts for each of the therapy groups and age matched controls. One way ANOVA revealed no significant differences between any of the treatment groups or control groups, but associated confidence intervals were wide.



Colony forming units for macrophages and granulocytes (CFU_{gm}) Counts

A number of assays failed as a result of technical problems and consequently colony data is limited to the 3.7 MBq x 2, 2.4 MBq x 3 and 1.8 MBq x 4 groups. Colony counts per 10⁵ plated mononuclear cells are shown with their respective age matched controls in Figure 4.14. One way ANOVA was used to look for differences in colony counts between any of the treatment groups (Figure 4.15). No significant differences were seen between any of the treatment or control groups ($p=0.795$, 0.865 respectively).

Photograph 4.1: Murine granulocyte macrophage colonies photographed using an inverted microscope. Two single colonies are shown with the second demonstrating florid growth giving the appearance of multiple colonies.

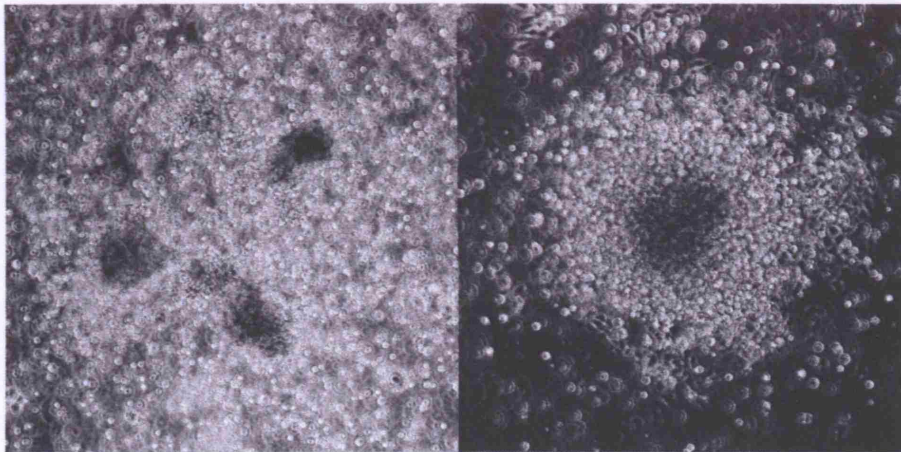


Figure 4.14: Mean colony counts alongside age matched controls. No significant differences were seen between any of the treatment groups or controls.

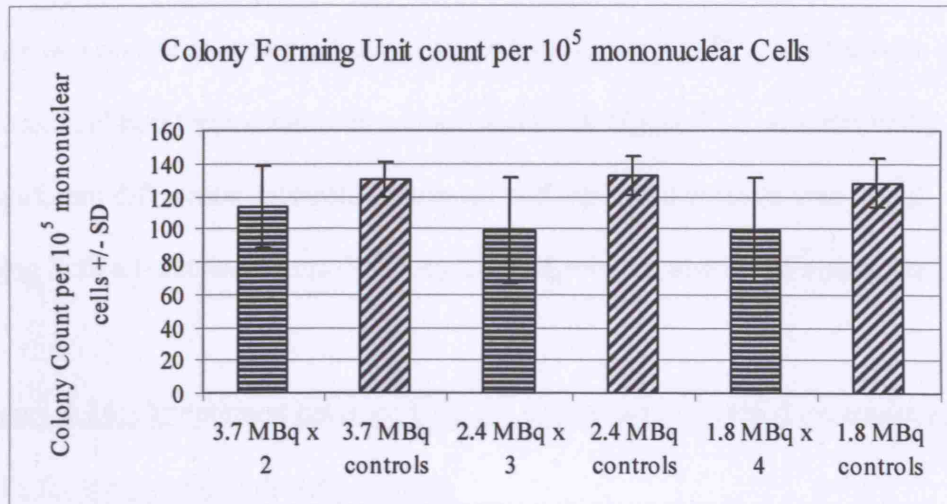
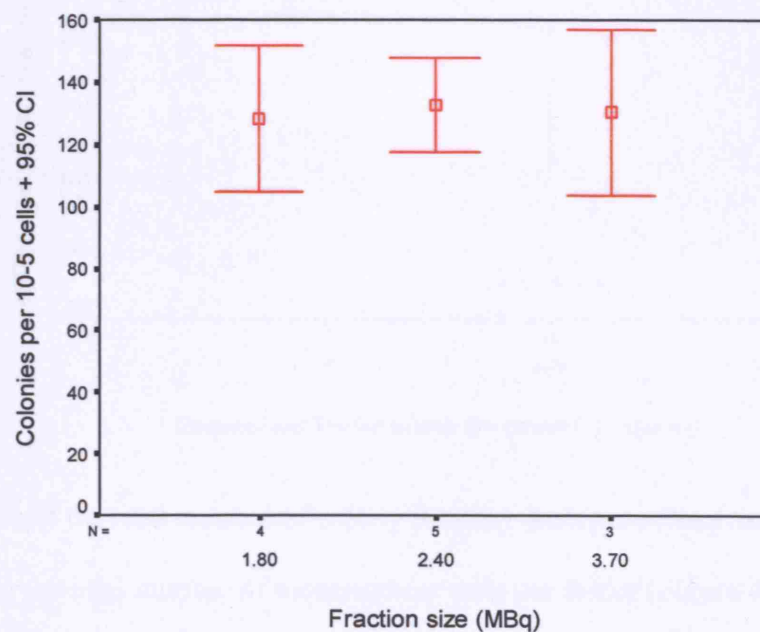


Figure 4.15: Mean colony counts per 10⁵ plated cells for each of the treatment groups + 95% Confidence intervals.

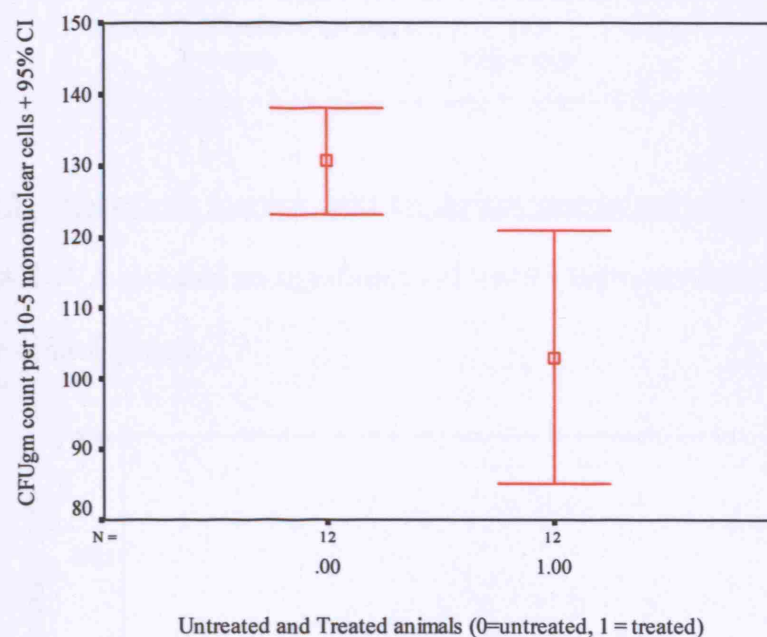


A Mann-Whitney U test was used to analyse differences between the colony counts in each of the treatment groups and their respective age matched

controlled and revealed no significant differences for the 3.7 MBq, 2.4 MBq or 1.8 MBq groups ($p=0.275$, 0.513 and 0.275 respectively).

Data was pooled together subsequently to look for any differences between the treated and non-treated animals and are shown in Figure 4.16. A statistically significant difference between the treated and untreated animals was found using both a t-test and Mann-Whitney U test ($p=0.005$ and 0.011 respectively).

Figure 4.16: Comparison between CFUgm counts per 10^5 plated mononuclear cells for treated and untreated animals



An estimate of the total number of colony forming units per animal was also made using the total number of mononuclear cells per femur (Figure 4.17).

Again no significant differences were seen between any of the therapy or control groups ($p=0.183$ and 0.154 respectively) or between the therapy groups 3.7 MBq x 2, 2.4 MBq x 3 and 1.8 MBq x 4 and their respective age matched controls ($p=0.827$, 0.076 and 0.083 respectively), as shown in Figure 4.18

Figure 4.17: Total femoral CFU_{gm} per mouse with associated age matched controls. No significant differences were detectable between treatment groups and their respective age matched controls using a Mann-Whitney U test.

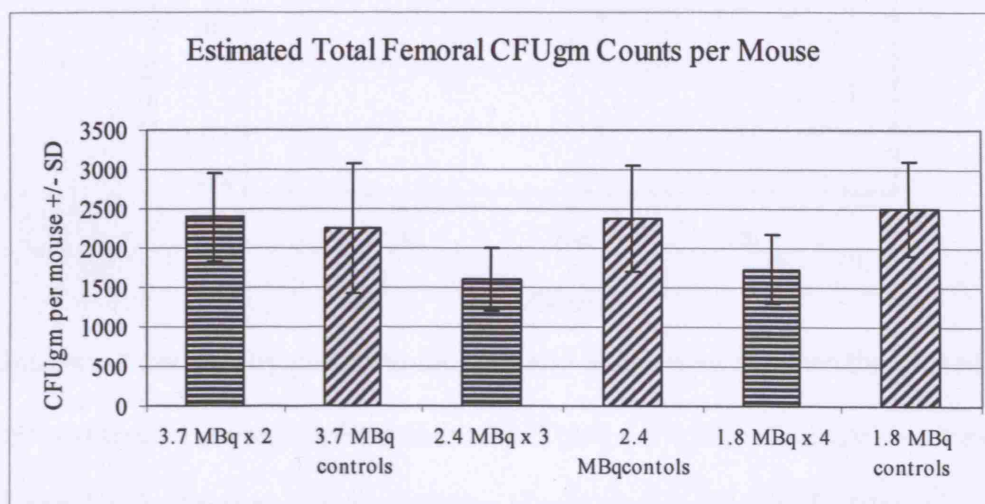
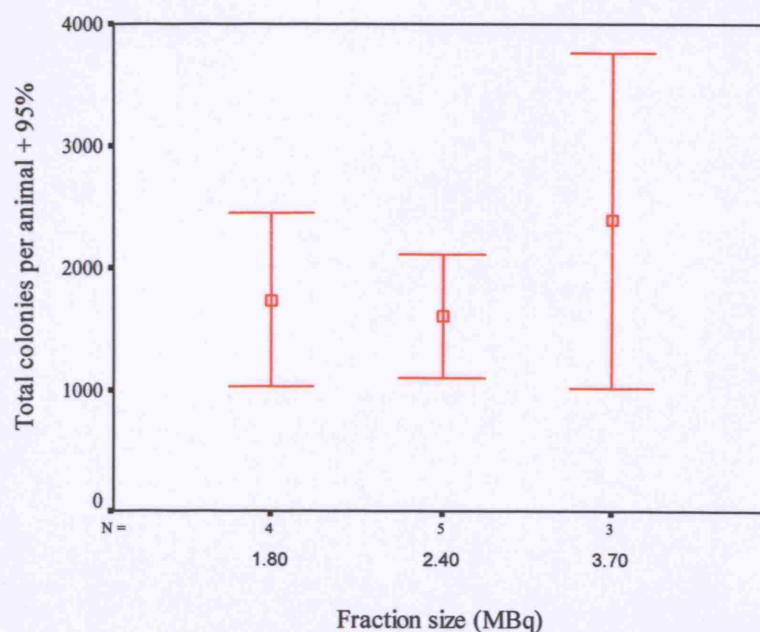
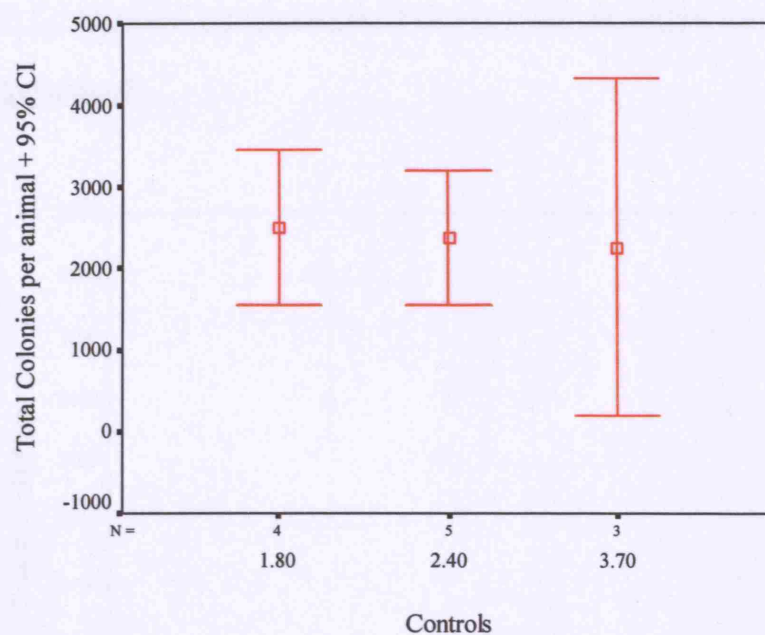


Figure 4.18: Total colony forming units for therapy groups and control groups.

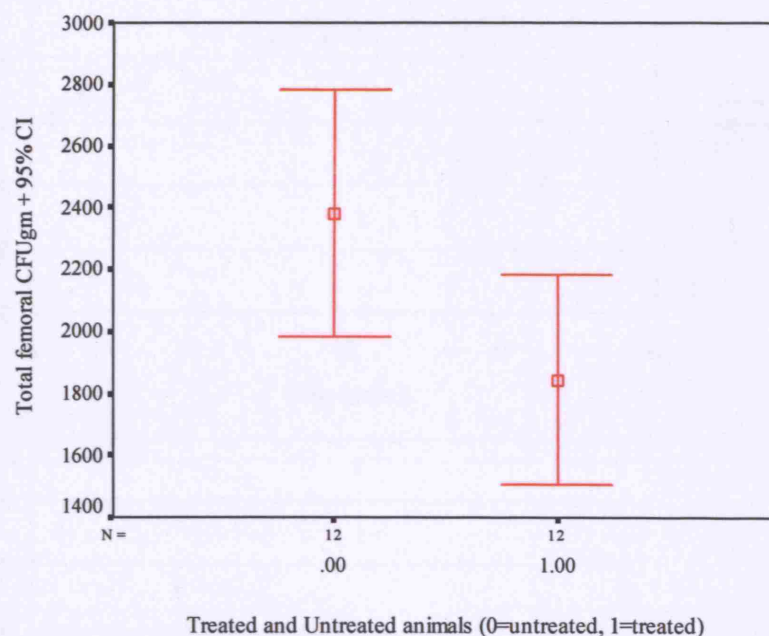
One way ANOVA revealed no significant differences between any of the therapy or control groups.





Data was subsequently pooled to look for any differences between the treated and non-treated animals and are shown in Figure 4.19. Statistical analysis was undertaken by t-test and Mann-Whitney U tests and revealed a significant difference between the treated and untreated animals ($p=0.034$ and $p=0.04$ respectively).

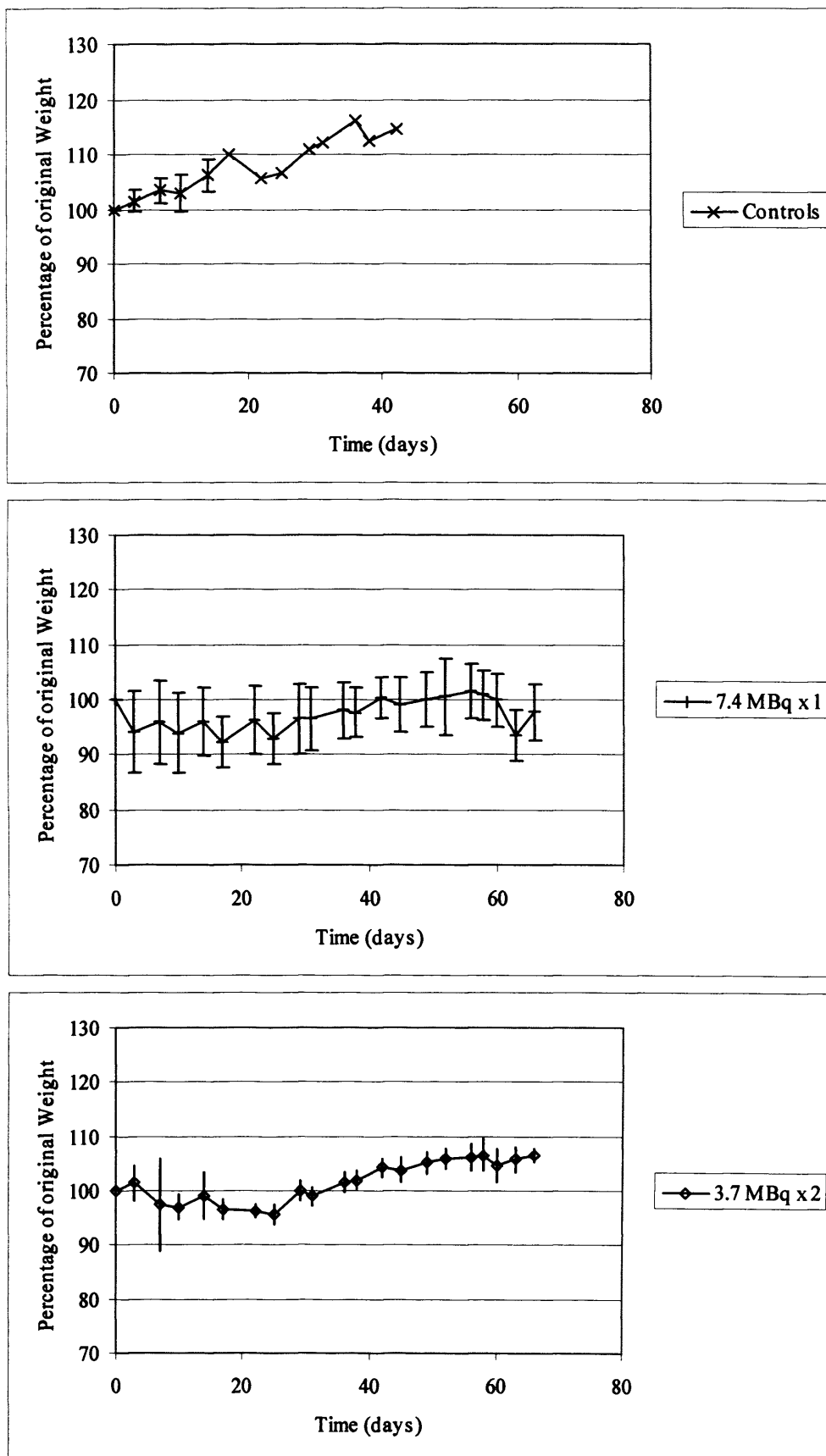
Figure 4.19: Comparison between total CFUgm counts per animal for treated and untreated animals

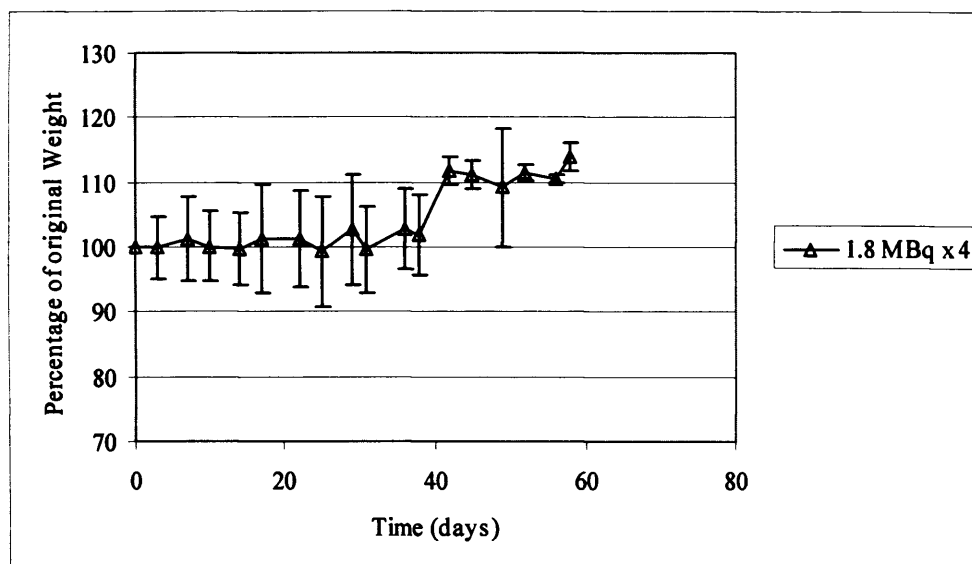
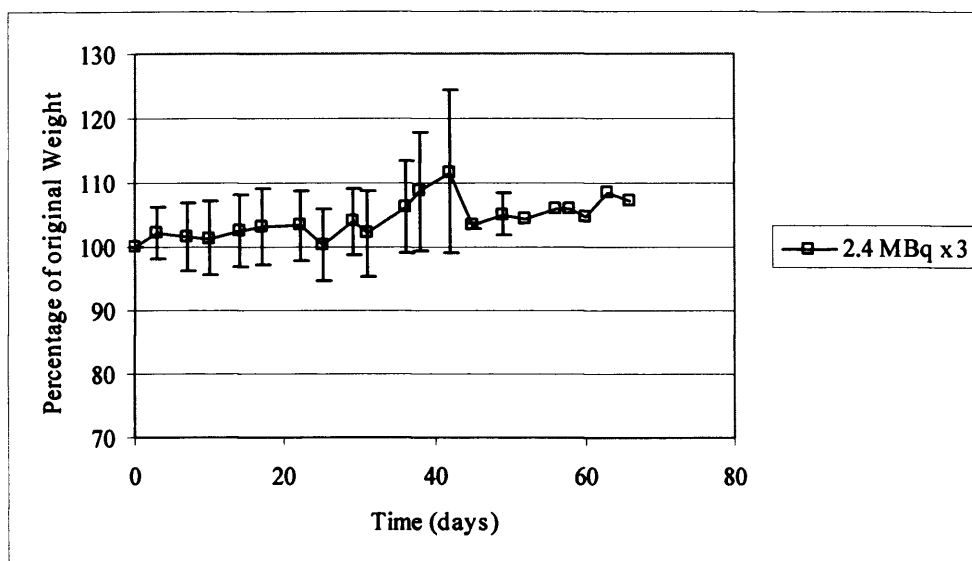


4.3.12 Systemic toxicity: Weight

Control mice gained weight steadily during the course of the experiment whereas weight gain was slower in all of the treatment groups (Figure 4.20). A fall of 10% in body mass was seen in two of the animals in the 7.4 MBq x 1 group, and a single animal in both the 3.7 MBq x 2 and 1.8 MBq x 4 groups.

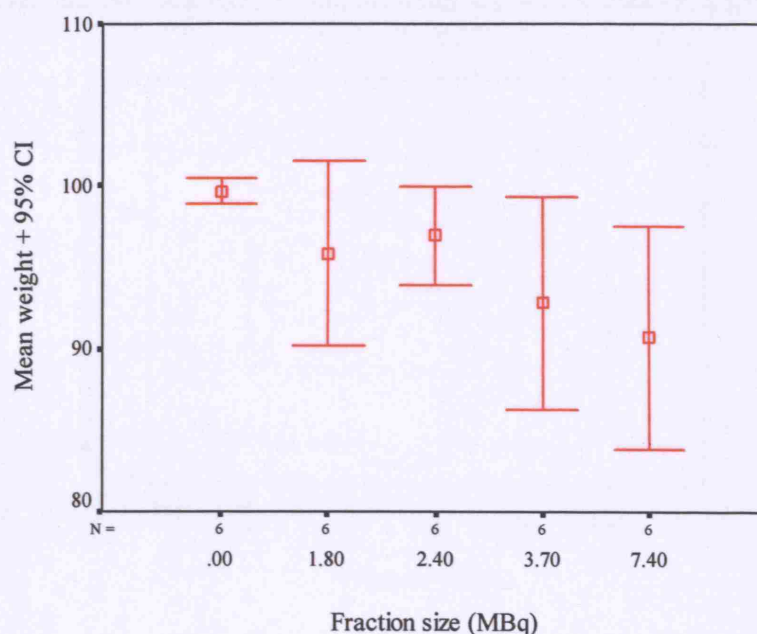
Figure 4.20: Mean animal weights as percentage of baseline (+/-SD).





The mean nadir weight for each group is shown in Figure 4.21 and analysis using a one way ANOVA revealed a significant difference between the groups ($p=0.033$). Ad hoc testing demonstrated a significant decrease in body mass for the 7.4 MBq group compared to controls ($p=0.039$), suggesting increased systemic toxicity in this group.

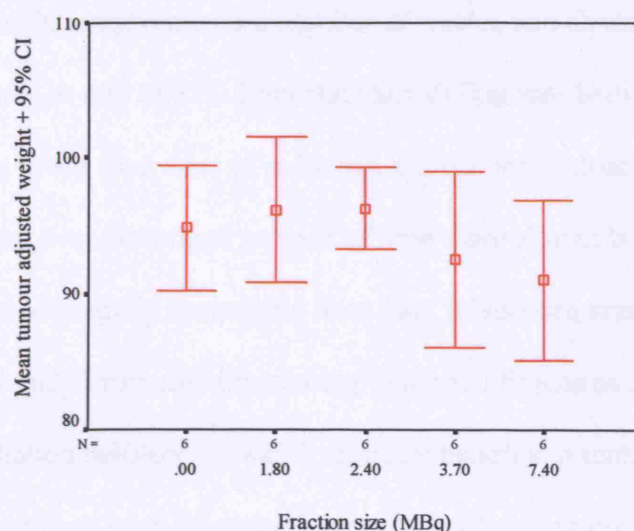
Figure 4.21: Mean nadir weights as a percentage of baseline (+/- 95% CI)



However, given the relatively large contribution of the tumour mass to the overall animal body mass, data was also analysed following correction for tumour (assuming a specific gravity of one). Using tumour adjusted weights, one animal in each of the groups experienced weight loss of 10% except for the 2.4 MBq x 3 group. Tumour-adjusted nadir weights for the treatment groups are shown in Figure 4.22 and a one way ANOVA revealed no significant differences between any of the treatment groups or controls ($p=0.291$).

Figure 4.22: Mean tumour adjusted nadir weights + 95% confidence intervals.

No significant differences were noted between any of the treatment groups.



4.4 Discussion

Current approaches in colorectal cancer using systemic chemotherapy cure only a small proportion of patients and are associated with significant patient morbidity. Despite intensive investigation, RIT of colorectal cancer has yielded only modest results and remains investigative. Due to the high incidence of neutralising antibody formation seen with murine antibodies in patients following even a single exposure, most RIT to date has been performed using single administration schedules. However with the advent of chimeric and humanised antibodies of reduced immunogenicity, repeated therapies with RIT are now possible.

Dose fractionation is established practice in EBRT and has been shown to lead to a reduction in late normal tissue toxicity allowing for dose escalation. In addition, during a typical six week course, significant tumour revascularisation is thought to occur which is thought to overcome the problem of hypoxic

radioresistance. Fractionation has also been proposed for RIT as a means of improving its therapeutic ratio. However, the radiobiology of daily bursts of high dose rate radiation given over a number of weeks, and characteristic of EBRT, is different to that of RIT. Important key differences between RIT and EBRT include a lower total dose of radiation, a much lower dose rate of radiation delivery over prolonged periods of time more akin to brachytherapy, and finally an exponentially decreasing dose rate. It has been argued therefore that RIT may already be maximally sparing to normal tissues as a result of the low rates of radiation delivery, in which case any benefits in terms of normal tissue sparing resulting from fractionation are likely to be minimal (433). In addition, the primary dose limiting organ in RIT is the red marrow and its progenitor cells, which behave as early responding tissues with limited repair capacity. As a result fractionation might be expected to make less difference to outcome, with total dose being far more important (140;321).

As a result from conventional radiobiology it would seem unlikely that further fractionation would allow for significant dose escalation. This is important as the linear quadratic model would predicts that radiation is less effective as the dose rate is lowered due to a dose rate effect (114;433-435). Furthermore the longer overall treatment times associated with fractionation will also be associated with increased tumour repopulation further reducing the efficacy of therapy.

It is perhaps surprising therefore that a number of preclinical studies have suggested that fractionation of RIT may lead to an enhancement of therapeutic

effect even in the absence of dose escalation (323;326-328). Proposed mechanisms for the increased efficacy of fractionated RIT have been reviewed by DeNardo et al and relate to unique features of the delivery of radiation by RIT (436). Fractionation may address the problem of heterogeneous antibody delivery in tumours by allowing subsequent doses of antibody to access different regions of tumour, provided that tumour shrinkage has led to reductions in interstitial pressure and the redistribution of blood flow (274;436). Additional benefits of such vascular re-distribution would also include re-oxygenation of hypoxic tumour regions, leading to increased radiosensitivity in subsequent therapies as occurs during fractionated EBRT. Pre-clinical studies, using serial sections autoradiography to reconstruct tumour activity, suggest that fractionated RIT may also lead to less dose rate uniformity over time in the well vascularised and actively growing tumour margins compared to single dose administrations (324;329). Another potential benefit of fractionated RIT relates to cell cycle redistribution effects. Both G1/S and G2/M phase transitions in the cell cycle are under close surveillance for the purposes of preventing replication of damaged DNA and G2/M phase arrest has been observed following the exponentially decreasing dose rate irradiation typical of RIT (437). Since G2/M is known to be a more radiosensitive phase of the cell cycle, it has been suggested that such mechanisms may lead to enhanced radiation sensitivity upon subsequent doses of radiation during fractionated RIT, depending upon the inter-fraction interval. In addition at the low doses and dose rates seen during RIT, it is possible that a low dose hyperradiosensitivity effect could be observed, offsetting the reduced efficacy one might expect by fractionating the same administered activity as a result of the dose rate effect and tumour repopulation.

In this study using a ^{131}I labelled A5B7 in nude mice bearing LS174T xenografts, a comparison of the effects of dose fractionation of RIT has been performed by administering the same total activity as either a single administration or as a number of separate administrations. With regards to tumour growth, although tumour growth delay was seen in all of the treatment groups compared to controls, fractionation appeared to have a clear detrimental effect upon long term cure of tumours. Cures were seen in 5/6 animals receiving the single fraction therapy and 2/6 animals receiving a bi-fractionated administration, while no cures were seen in either the 3 or 4 fraction groups. Previous work using the F(ab')_2 fragment of A5B7 in the same xenograft model system comparing the single administration of antibody to administration of the same activity as three separate fractions has similarly suggested reduced efficacy as a result of fractionation (246). This data suggests that in this tumour model system and with the fractionation regimes chosen, that dose rate effects and repopulation are perhaps more important than the other factors thought to contribute to the improved efficacy of fractionated RIT outlined above. Another possible explanation for the discrepancy seen with other published data is that the total dose of radiation delivered to tumour sites in our fractionated regime was less than that achieved by a single administration. Modest reductions in CEA binding of A5B7 over the course of therapy are evident by Day 7 in vitro and may have reduced the efficiency of targeting, hence reducing the total dose. Furthermore there were also modest falls in radiopharmaceutical purity, with a progressive decrease in the proportion of bound to free antibody over the course of administration, something that may also be expected to reduce the tumour absorbed dose. However the size of these changes appears

modest in comparison with the difference in tumour control seen in the study. In addition reduced vascular permeability as a result of prior radiation exposure has also been noted 7 - 21 days following RIT in other tumour model systems and has been found to lead to reductions in tumour uptake of a second dose of radiolabelled antibody (282;331). Although such changes have been shown to be tumour-type dependent, the LS174T xenograft has reliably demonstrated a reduced vascular permeability as a result of RIT (332). Additional studies looking directly at tumour absorbed dose would answer the question of whether reduced absorbed dose as a result of fractionation were an important factor here.

In terms of the therapeutic ratio, however, if a significantly reduced incidence of haematological toxicity were observed following fractionated RIT, dose escalation might be possible to overcome any loss of tumour kill. Pre-clinical studies suggest that fractionated approaches may be less myelotoxic than single therapy regimes (322;326;327). In the study by Beaumier et al, delayed and incomplete recovery of WBC counts was seen following single administration of therapy whereas this was not seen following administration of a multifraction regime of equivalent administered activity (322). In the study by Schlom et al, marrow aplasia resulted from a single administration of radiolabelled antibody, whereas animals receiving the equivalent administered activity in a fractionated manner avoided this complication (326). However the most comprehensive study of the effects of fractionated RIT on haematological toxicity has been undertaken in non-tumour bearing animals (327). Acute haematological toxicity was assessed by daily blood counts, and an assessment of late marrow toxicity made by assaying early marrow progenitor cells using CFUgm assays. In this

study, single injections of RIT were associated with earlier and more severe haematological toxicity (thrombocytopenia and granulocytopenia) and lower bone marrow CFU_{gm} counts.

In the current study an assessment of alterations in acute haematological toxicity is difficult as the amount of acute blood toxicity observed was limited.

However, despite this there were no significant differences between single or fractionated therapies for any of the acute haematological indices. In addition following therapy no differences in early progenitor cell numbers were seen between any of the treatment groups. Although there was a trend to lower CFU_{gm} counts in each of the therapy groups compared to controls, this was not significant on Mann-Whitney U test. However this negative finding might be explained by the small number of data points available; pooling the data together to compare treated and non-treated animals demonstrated a significant depression of CFU_{gm} function by RIT

In relation to systemic toxicity, which was assessed using whole animal weights, a significant increase was suggested in mice undergoing a single administration of radiolabelled antibody compared to those undergoing fractionated therapy. However when animal weights were corrected for tumour mass, no difference was evident.

The limited overall toxicity seen here may reflect the low total administered activity used, which was chosen following preliminary studies showing that 18.5 MBq of ¹³¹I A5B7 produced significant tumour control whilst also causing a

degree of haematological toxicity (Figure 5). However the majority of reported pre-clinical studies of RIT have used higher, often myeloablative activities, and then derived fractionated therapy schedules using this as a starting point. Published data on the behaviour of marrow progenitor cells have shown that they have a high ability to repopulate as long as marrow ablation does not occur, and it is possible that this may be responsible for the marrow sparing effects of fractionation seen in other studies.

4.6 Conclusions

For a given total administered activity in this tumour model system, fractionation reduced efficacy. This is not surprising given that in the absence of activity escalation reduced tumour cell kill as a result of lower dose rates and a prolonged overall treatment time, allowing repair of DNA damage and proliferation of tumour cells between fractions, might be expected. This suggests that in this tumour model system other radiobiological considerations unique to RIT such as the inverse dose rate effect, tumour revascularisation and improved delivery of radiation to actively growing areas of tumour are unable to overcome the effects resulting from the dose rate effect and tumour repopulation. Although the ability to dose escalate as a result of fractionation was not directly addressed in these experiments, systemic toxicity as assessed by weight revealed little evidence of reduced toxicity following fractionation. Although there appeared to be more weight loss in the single fraction group compared to the multi-fractionated groups, this did not persist when tumour weight was accounted for. In addition, fractionation was not associated with any reduction in either acute blood toxicity or early marrow progenitor cell function,

although this may reflect the wide confidence intervals associated with this data due to the small number of subjects. Should these findings be confirmed, they suggest that fractionation of therapy using a fixed administered activity of radiation does not improve the therapeutic effect of RIT. Whether fractionation would allow for significant dose escalation by reducing normal tissue, and in particular marrow toxicity has only been indirectly addressed in this study. However, firm conclusions regarding changes in haematological toxicity are not possible given the lack of data, although systemic toxicity as assessed by changes in body weight suggests little benefit as a result of fractionation.

Acknowledgements

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My contribution was in the planning of the experiments, performance of the CFU_{gm} assays and the day to day management of the animals following treatment, including assessment of tumour response to therapy. I am primarily responsible for the interpretation of the data and the conclusions drawn from them.

<u>Contributor</u>	<u>Contribution</u>
Dr Barbara Pedley	Planning of experiments, principal supervisor of work
Dr Jason Dearling	Radiolabelling of antibody, quality control procedures during experiment
Robert Boden	Passaging, phlebotomy, animal husbandry
Dr Steve Marley	Teaching of CFU _{gm} assay technique, interpretation of CFU _{gm} data
Dr Letizia Feroni	Manual blood counting, interpretation of CFU _{gm} data Use of automated haemocytometer for FBC measurements

Overall Conclusions

5.1 Background

The three hypotheses that have been addressed in this thesis are:

- A. That radioimmunotherapy with ^{131}I -CHT-25 will be an effective treatment modality of low immunogenicity for patients with IL-2R expressing lymphomas.**
- B. That the adoption of individual patient dosing based upon tracer studies may potentially improve therapeutic outcomes following RIT using ^{131}I -CHT-25 in patients with relapsed/ refractory IL-2R expressing lymphomas.**
- C. That fractionation of Radioimmunotherapy using ^{131}I -A5B7 in a colorectal tumour xenograft model will lead to an improvement in the therapeutic ratio of treatment.**

These hypotheses will be discussed in detail below.

5.2 Radioimmunotherapy with ^{131}I -CHT-25 is an effective treatment modality of low immunogenicity for patients with IL-2R expressing lymphomas.

Lymphomas have proved an attractive target for RIT due to their expression of a large number of well-defined surface antigens, an increased susceptibility to immunomodulation and their high radiosensitivity. The IL-2R α is an attractive target for RIT given that it has a limited physiological expression. It is also abnormally expressed upon a number of different tumour types including HTLV associated adult T-cell leukaemia, T-cell lymphoma and Hodgkin's disease. Furthermore the IL-2R α has already proved a useful antigen for antibody-directed therapies and has been successfully targeted using both naked and radiolabelled whole antibody (296;438) and using a single chain Fv fragment fused to a truncated form of *Pseudomonas* exotoxin (81). Therefore the use of ^{131}I -CHT-25, a chimeric antibody targeting the receptor, has been proposed as a novel treatment of low immunogenicity for IL-2R-expressing lymphomas. In order to investigate this hypothesis, data has been presented in Chapter 2 following the administration of ^{131}I -CHT-25 to nine patients with refractory/relapsed lymphoma in a phase I/II dose escalation study.

In RIT, infusional related toxicity is usually allergic in nature and this has been mild using ^{131}I -CHT-25, comprising only CTC grade 1 fever and nausea and fatigue in a few patients. The observation that no serious infusion-related toxicity or anaphylactic episodes have occurred despite multiple administrations of antibody in a number of patients is indicative of low immunogenicity and this

is confirmed in Chapter 3 with the presentation of formal HAMA titres. In keeping with other studies of RIT the main toxicity observed following therapy has been delayed haematological toxicity. This has been observed in a dose-dependent fashion but with CTC grade 3 and grade 4 toxicities (with the exception of lymphopenia) only becoming apparent at the 1480 MBq/m² dose level and above. Moreover the cytopenias observed in patients treated at the highest dose levels have often also shown a degree of reversibility. In this regard, it is interesting that little difference in haematological toxicity was seen when either administered activity or cumulated administered activity were correlated with haematological toxicity, suggesting that an element of marrow repopulation might be occurring. This is in keeping with the known radiobiology of marrow stem cells.

Only a single patient receiving a cumulative activity of greater than 10 GBq, and in whom the final treatment was administered at the highest dose level of 2960 MBq/m², failed to regain adequate marrow function. A single toxic death was observed in this patient who failed to engraft following infusion of stem cells and subsequently died following development of *Pneumocystis carinii* pneumonia. Following this the highest dose level was removed from the protocol and all subsequent patients commenced therapy at the 740 MBq/m² dose level. Although the non-myeloablative MTD is still not established, it is anticipated from the experience gained so far that this lies between 740 and 1480 MBq/m². Other non-haematological toxicity has been mild and included hypothyroidism requiring thyroxine replacement in a single patient and transient alterations in liver function tests.

In terms of treatment responses, it is encouraging that evidence of efficacy has already been seen in this heavily pre-treated group of patients. Using FDG, PET responses have been seen at all dose levels from 370 to 2220 MBq/m². In addition, CT responses have been seen in two patients following administered activities of 740, 1480 and 2220 MBq/m².

Although it is difficult to draw firm conclusions from the trial so far, given the small number of patients seen, the observation of significant therapeutic effects using ¹³¹I-CHT-25, in the absence of significant toxicity or the need for haematopoietic stem cell support, are a strong argument in support of the stated hypothesis.

In terms of future development of ¹³¹I-CHT-25, a number of modifications have been made to the clinical trial protocol. As the clinical trial was originally designed, repeated therapy would continue until disease progression, tumour eradication or myeloablation occurred. As a consequence, in practical terms all patients coming onto the study needed to have undergone a marrow or stem cell harvest. This requirement has proved a significant barrier to trial recruitment and is thought to have contributed to the slow accrual seen to date, which has averaged only three patients a year over the course of the study. However since activity has now been demonstrated at a non-myeloablative dose level, the future goal of the trial will be to determine the non-myeloablative MTD so that stem cell harvesting will no longer be a requirement for entry onto the study. In the most recent update to the trial protocol, therefore, future patients will receive up to two separate administrations of radiolabelled antibody at an administered

activity of 1200 or 1480 MBq/m² in order to define the non-myeloablative MTD.

An additional problem that has been encountered with the current protocol is a difficulty in assessing toxicity as patients could be re-treated at a 1-month interval. Since this is often around the time of the nadir in blood indices following the previous therapy, it has been difficult to know whether subsequent toxicity related to initial or subsequent exposures to ¹³¹I-CHT-25. In order to avoid this, future patients will be re-treated following an interval of at least six weeks.

5.3 Individual patient dosing based upon tracer studies may potentially improve therapeutic outcomes following RIT using ¹³¹I-CHT-25 in patients with relapsed/ refractory IL-2R expressing lymphomas.

The use of ¹³¹I as a therapeutic radionuclide has the advantage of a γ emission that can be used during the development of novel RIT agents to perform detailed biodistribution studies. In the first part of Chapter 3 I have investigated the effects of chimerisation upon the biodistribution and dosimetry of ¹³¹I-CHT-25 by comparing it to that of ¹³¹I-A5B7. Both antibodies are currently prescribed depending upon body surface area, with dose escalation studies used to derive the maximum tolerated dose for use in future patients. However it has been observed for many ¹³¹I labelled antibodies that considerable variability in biodistribution may occur between patients. As a consequence, ¹³¹I-

Tositumomab is currently prescribed on an individual patient basis, depending upon biodistribution studies performed prior to therapy.

I have subsequently used the biodistribution data for ^{131}I -CHT-25, along with haematological toxicity data, to address the hypothesis that individual patient dosing methods based upon tracer studies may be a better way to deliver therapy with this antibody.

An interesting difference between ^{131}I CHT-25 and the two commercially available antibodies for the treatment of B-cell NHL, ^{131}I -Tositumomab and ^{90}Y Ibritumomab, is its chimeric structure. Although chimeric antibodies are reported to have reduced immunogenicity compared to their murine counterparts, they have been less commonly used in RIT because of concerns that their extended circulating half-life might lead to enhanced normal tissue toxicity. The biodistribution studies presented in Chapter 3 have demonstrated that ^{131}I -CHT-25 is associated with a markedly prolonged circulating half-life in comparison to A5B7; the recorded whole body activity half-life for the former is 66.9 hours in comparison to the latter which is only 26.9 hours. As a direct consequence of this a lower administered activity of ^{131}I -CHT-25 is required to deliver an equivalent dose of radiation to bone marrow than ^{131}I -A5B7 and since this is dose limiting in non-myeloablative RIT, it will affect their respective patterns of radiation delivery. In particular this means for any given marrow absorbed dose ^{131}I -CHT-25 will deliver radiation at a lower dose rate and for a longer length of time than ^{131}I -A5B7 i.e. in a more fractionated manner. How this might affect the therapeutic ratio of treatment is an important consideration and difficult to answer given that the effects of

increasing fractionation upon the response of normal tissues and tumour are complex. However, in the currently reported phase I/II study using ^{131}I -CHT-25, the fractionated nature of radiation delivery with ^{131}I -CHT-25 has not precluded significant therapeutic effects, though whether use of the radioiodinated murine parent of CHT-25 would have led to an improved therapeutic ratio is unknown.

One might speculate that increasing fractionation could be a therapeutic barrier in the more common solid malignancies in which tumour kill shows a greater dependence upon dose rate and may be an important factor in explaining the apparent reduction in efficacy of fractionated RIT seen in Chapter 4. It could also be argued however that the lower immunogenicity of chimeric antibodies allows for multiple therapies to be administered, and that this might offset any potential reduction in efficacy as a result of lower tumour dose rates. With regard to this, HAMA rates reported in Chapter 3 reveal only a single anti-idiotypic response to ^{131}I -CHT-25 following 18 administrations of antibody. This is in comparison to ^{131}I -A5B7 in which 6/7 administrations were associated with the development of HAMA positivity.

Looking at the biodistribution of ^{131}I -A5B7 and ^{131}I -CHT-25, in common with other ^{131}I labelled antibodies, considerable variation in biodistribution patterns has been seen. As a result, it is unlikely that the use of a single dose based upon body surface area will deliver a reproducible dose of radiation to normal tissues. In addition, however, data from individual patients undergoing repeated therapies suggests that the degree of variability in biodistributions for any single patient undergoing repeated exposures is small. This suggests that tracer studies

could accurately predict the biodistribution of subsequently administered therapeutic antibody.

The importance of developing a dosing regime that delivers a reproducible dose to normal tissues is primarily related to safety, in that only by knowing *a priori* the absorbed dose in normal tissues can one predict the likely normal tissue toxicity for any given administered activity. Given this I have compared the ability of MIRD absorbed dose estimates, which gives an average absorbed dose per administered activity for the study population, to that of simple but individual biodistribution parameters known to influence marrow dose, namely whole body AUC and blood activity AUC. Using linear regression I have demonstrated that both these latter indices, which can be simply measured, are as good as or better than the MIRD absorbed dose in predicting blood toxicity. This suggests that adopting an individualised approach to patient dosing might improve the consistency of therapy using ^{131}I -CHT-25, assuming that observed levels of haematological toxicity are related to marrow absorbed dose.

In the technique of Wahl et al, the use of whole body absorbed dose to predict marrow absorbed dose given with ^{131}I labelled antibodies is reasonable given that a high proportion of the marrow absorbed dose depends upon the whole body component. However this type of approach is not possible for therapy using radionuclides in which there is no γ component such as ^{90}Y . A more generally applicable and accurate estimate of therapy absorbed dose following tracer studies could be achieved by applying individual patient dosimetry techniques during tracer studies to take account of all sources of radiation

affecting the red marrow. For an individual patient this would of course improve the ability of the tracer study to determine therapy doses, but in the longer term would additionally allow for the construction of more accurate normal tissue and tumour dose response curves following RIT. The construction of these would be a valuable first step towards the development of highly accurate dosimetry-led RIT of the level currently achieved during EBRT. In order to perform individual patient dosimetry, a number of improvements in both the gathering of activity data and subsequent data analysis would be required. The former could be addressed using CT-SPECT, which provides a true picture of the heterogeneous activity biodistributions following RIT in conjunction with anatomical data. In terms of data analysis a number of individual patient dosimetry methods have been described by different groups including Monte-Carlo and beta point source function techniques (439-445).

5.4 An Investigation of the effects of dose fractionation upon tumour growth and early and late haematological toxicity in nude mice bearing LS174T human colorectal xenografts treated with ^{131}I labelled monoclonal antibody A5B7

Despite the success of RIT in the therapy of NHL, there is a need to improve response rates in solid tumours. In the clinical treatment of colorectal cancer for example a large number of clinical studies have been undertaken and the success of treatment has been limited in comparison to lymphoma (422).

In external beam radiotherapy, fractionation has a specific meaning, which is the dividing up of the total dose into a number of smaller fractions. In this setting fractionation is a standard approach that has been shown to improve the therapeutic ratio. With the advent of antibodies of reduced immunogenicity it has become possible to deliver RIT repeatedly and it has been proposed that RIT may similarly benefit from fractionation. However extrapolation of the benefits of fractionation in EBRT to RIT is difficult given the significant differences between the two. EBRT delivers bursts of high dose rate radiation homogeneously to well defined normal tissue and tumour volumes, with the amounts of radiation that can be safely delivered being limited by toxicity in late responding normal tissues. By contrast RIT delivers continuous and exponentially decreasing low dose rate irradiation which is frequently heterogeneous, and although targeted, is dose limited by systemic toxicity. In addition the dose limiting normal tissue is not late responding, but acute responding red bone marrow.

Using radiobiological principles to predict the effects of fractionation upon the therapeutic efficacy of RIT is complicated. In terms of tumour kill one might expect that RIT, which is already highly fractionated, might be less efficacious at eradicating tumour due to the dose rate effect unless significant dose escalation were possible. Moreover since marrow progenitor cells are acutely responding one would predict that increasing fractionation would not be associated with significant reductions in toxicity. However there are additional factors to consider in radiation delivery by RIT given its dependence upon antibody biodistribution. Vascular changes in tumour which reduce the uptake

of successive administrations of antibody have been observed following repeated RIT which could further reduce the efficacy of RIT. On the other hand other consequences of fractionation might be beneficial. The reduction in effectiveness as the result of the dose rate effect might be overcome by the 'inverse dose rate effect' seen at the very low dose rates typical of RIT. Given the short treatment times of RIT, fractionation of therapy might allow a greater time for tumour shrinkage to occur during therapy and by leading to revascularisation and re-oxygenation; this might improve antibody biodistribution and overcome hypoxic radioresistance. In addition, fractionation has been shown to improve radiation delivery to the perivascular, actively growing areas of tumour.

However, perhaps the most compelling evidence for the use of fractionation comes from pre-clinical studies, which have demonstrated both increased tumour kill and/or reduced normal tissue toxicity, allowing for significant dose escalation. In addition these studies have suggested that fractionated RIT may be more effective than single administrations of therapy even in the absence of dose escalation. In Chapter 4 therefore, using a pre-clinical tumour model system, I have addressed the hypothesis that dose fractionation may improve the therapeutic ratio of RIT for a given administered activity.

In this investigation fractionation was not associated with any improvement in therapeutic efficacy and instead was associated with a reduction in both tumour control and cure. These findings are in accordance with previous work undertaken in the same tumour model system comparing single or fractionated

therapy using the F(ab')₂ fragment of A5B7 (246). These results suggest that any alterations in the balance of biological and radiobiological factors as a result of fractionation were not associated with enhanced anti-tumour effects in this model system. One important difference between this tumour model system and the data presented in Chapter 3 and clinical trials using radiolabelled Rituximab, in which efficacy has been seen even with the increasingly fractionated RIT offered by these chimeric antibodies, is that colorectal tumours have a lower α/β ratio than lymphomas. This will result in increased tumour sparing resulting from an enhanced dose rate effect. In addition, the 'inverse dose rate effect', which is thought to enhance the efficacy of RIT and compensate for tumour sparing at very low dose rates, has been much more commonly seen in lymphoma, and its relative lack in solid tumours may be another important factor responsible for the loss of efficacy seen in these experiments.

However fractionation could still be advantageous to RIT if it could be documented that it was associated with reduced haematological toxicity that might allow for safe dose escalation. In this study it has not been possible to demonstrate any detriment in either acute blood counts or early progenitor cell function as a result of fractionation. However it was more difficult than anticipated to assess the effects upon acute blood toxicity since so little was observed in any of the treatment groups. This was unexpected since the total therapy dose was chosen on the basis of preliminary investigations in which it led to measurable white cell toxicity. In addition, it has also been difficult to draw firm conclusions from the early progenitor cell function assays given the low numbers successfully performed, although the pooled data shows that the

irradiated mice as a group had lower counts than the non-irradiated group.

Finally an additional problem was that given the rapid tumour growth in the untreated mice, there was loss of most of the control group after Day 8.

Interestingly, these results as they stand appear to be at odds with a number of pre-clinical studies in which fractionation was observed to reduce acute blood cytopenias and early progenitor cell function. However an important difference is that almost universally in these studies a known myeloablative dose was chosen and then fractionated. It is perhaps not surprising therefore that fractionation is of benefit in these cases since marrow ablation is non-recoverable, whereas even with limited progenitor cell survival, considerable marrow recovery might still be possible through repopulation. By contrast, in this study a non-myeloablative total administered activity was chosen and fractionated. It is perhaps more unlikely that this would reveal differences between the treatment groups given the remarkable ability of the marrow to repopulate and also up-regulate mature cell production following radiation insults. Furthermore, since it is known that bone marrow has only a limited repair capacity, the total radiation-induced DNA damage following similar absorbed doses is unlikely to be affected greatly by increasing fractionation. The fact that fractionation is not associated with any reduction in marrow toxicity is supported by a recent clinical study in which fractionation of RIT using a similar inter-fraction interval to that chosen in this preclinical study has been of little benefit in reducing haematological toxicity (446). These results therefore offer limited support for fractionation of a given dose of radioactivity as a method to improve the efficacy of RIT. However a significant weakness in the data

presented here is that the experiments do not directly address whether fractionation would allow for a significant increase in administered activity which may enhance the therapeutic ratio. Incorporation of fractionated groups in which the administered activity were increased to account for losses of efficacy as a result of increased re-population and the dose rate effect were originally planned as part of the therapy protocols, but omitted due to resource constraints.

5.5 **Future work:-**

Clinical Studies

Complete dose escalation of phase I/II study using ^{131}I -CHT-25 in patients to define MTD.

Document efficacy of ^{131}I -CHT-25 in patients with IL-2R expressing lymphomas treated at the MTD.

Implement individual patient dosimetry for patients treated with ^{131}I -CHT-25 and construct algorithms relating whole body absorbed dose and blood AUC to marrow absorbed dose. See Appendix 2 for preliminary work relating to the potential adoption of individual patient dosing depending upon whole body clearance times as described by Wahl et al (378).

Pre-Clinical Studies

Perform detailed tumour biodistribution studies following single administration or multiple dosing regimes to determine if reduced total antibody accumulation is responsible for decreasing tumour control as a result of fractionation. In addition these experiments would also be used to determine the increase in activity required by the fractionated therapies in order to overcome dose rate and repopulation effects; this information will be obtained with the assistance of an experienced radiobiologist. On the basis of these experiments, plan further experiments delivering fractionated RIT to deliver an equivalent 'effective' radiation dose to tumour and also assess acute marrow toxicity and early

progenitor cell function with the aim of directly answering whether significant dose escalation is possible as a result of fractionation.

Given the problems in assessing haematological toxicity due to animal mortality, it may be necessary to repeat toxicity studies:

In non-tumour bearing mice to ensure effective controls are present

In a greater number of mice to reduce confidence intervals

Publications and Awards in Support of the thesis

Awards

Cancer Research UK clinical research fellow 2002-2005

Abstracts

J.A.Violet, R.Francis, J.R.Buscombe, J.Croasdale, P.Amlot, R.H.J.Begent. A Dose-Escalation-Study Evaluating the Toxicity and Therapeutic Efficacy of ^{131}I labelled Chimeric Antibody CHT-25 in Patients with IL-2 Receptor Expressing Lymphomas: Preliminary Results. Society of Nuclear Medicine 51st Annual Meeting

J.A.Violet, J.L.J Dearling, S.J Marley, A.J Green, R.H.J. Begent, R.B.Pedley. Dose fractionation reduces efficacy of radioimmunotherapy without decreasing toxicity. Annual Congress of the European Association of Nuclear Medicine, Istanbul, Turkey, October 2005. European Journal of Nuclear Medicine and Molecular Imaging, 32(S1): S207.

J.A.Violet, J.L.J. Dearling, S.J. Marley, A.J. Green, R.H.J. Begent, and R.B. Pedley. Fractionated radioimmunotherapy is less effective without decreasing toxicity. National Cancer Research Institute Conference, Birmingham, October 2005

J.A.Violet, J.L.J. Dearling, A.J. Green, R.H.J. Begent. Correlation of Biodistribution Data and MIRD Absorbed Dose Estimates to observed Haematological toxicity in Clinical Radioimmunotherapy (RIT) using ^{131}I CHT-25. National Cancer Research Institute Conference, Birmingham, October 2005

Appendix 1: SPECT and Dosimetry Calculations

Table A.1: Data spreadsheet for SPECT analysis

[illegible]

Table A.2: Data spreadsheet for Regions of interest definition

Thorax Data					Abdomen Data				
Time and Date of scan					Time and Date of scan				
29/Mar/2004 18:08					29/Mar/2004 17:41				
3.4					3.0				
Lung	slice	total counts	number of pixels		slice	total counts	number of pixels		
	33	35110.2	63						
	33	39018.8	88						
	38	42920.2	80						
	Sum	117049.2	231		Sum	0	0		0
	mean c.p.p		506.7		mean c.p.p				
Mean c.p.p per second					Mean c.p.p per second				
20.3									
Heart	slice	total counts	number of pixels		slice	total counts	number of pixels		
	29	94108.5	64						
	31	160483.9	110						
	33	120571.2	81						
	Sum	375163.6	255		Sum	0	0		0
	mean c.p.p		1471.3		mean c.p.p				
Mean c.p.p per second					Mean c.p.p per second				
58.8									
Liver	slice	total counts	number of pixels		slice	total counts	number of pixels		
					67	121019.9	108		
					64	89035	77		
					62	68995.4	56		
	Sum	0	0		Sum	279050.3	241		
	mean c.p.p				mean c.p.p.		1157.9		
Mean c.p.p per second					Mean c.p.p per second				
					46.3				

Appendix 1: SPECT and Dosimetry Calculations

Table A.1: Data spreadsheet for SPECT analysis

Treatment Details

Patient Trial Identification number	09
Injected Activity (MBq)	1093.5
Date of injection	29-Mar-04
Time of injection	14:14:00
Start time	14:42:00
Finish time	

Half-life of Radioisotope (iodine)	192.48
SPECT Correction factor (m)	0.0021

Thorax Scanning Details

Scan Date	Scan Time	Time following injection	Acquisition Time
29/03/2004	18:08	3.4	25
30/03/2004	15:11	24.5	25
31/03/2004	11:11	44.5	25
01/04/2004	15:47	73.1	25
02/04/2004	10:23	91.7	25
05/04/2004	10:36	163.9	30

Abdomen Scanning Details

Scan Date	Scan Time	Time following injection	Acquisition Time
29/03/2004	17:41	3.0	25
30/03/2004	14:43	24.0	25
31/03/2004	10:41	44.0	25
01/04/2004	15:19	72.6	25
02/04/2004	09:57	91.3	25
05/04/2004	10:06	163.4	30

Table A.3: Data spreadsheet for biodistribution of radioactivity

	Date and Time of scan	Time (hours)	c.p.p per sec	Activity per ml	Fractional activity/kg
Lung	29/Mar/2004 18:08	3.43	20.27	0.04	0.04
	30/Mar/2004 15:11	24.48	15.48	0.03	0.03
	31/Mar/2004 11:11	44.48	11.29	0.02	0.02
	01/Apr/2004 15:47	73.08	7.98	0.02	0.02
	02/Apr/2004 10:23	91.68	6.91	0.01	0.01
	05/Apr/2004 10:36	163.90	2.41	0.01	0.00
Heart	29/Mar/2004 18:08	3.43	58.85	0.12	0.11
	30/Mar/2004 15:11	24.48	45.00	0.09	0.09
	31/Mar/2004 11:11	44.48	37.91	0.08	0.07
	01/Apr/2004 15:47	73.08	28.50	0.06	0.05
	02/Apr/2004 10:23	91.68	22.44	0.05	0.04
	05/Apr/2004 10:36	163.90	8.40	0.02	0.02
Liver	29/Mar/2004 17:41	2.98	46.32	0.10	0.09
	30/Mar/2004 14:43	24.02	32.34	0.07	0.06
	31/Mar/2004 10:41	43.98	26.94	0.06	0.05
	01/Apr/2004 15:19	72.62	22.97	0.05	0.04
	02/Apr/2004 09:57	91.25	19.58	0.04	0.04
	05/Apr/2004 10:06	163.40	9.40	0.02	0.02

Table A.4: Data Spreadsheet for Monoexponential modelling of lung activity data using excel solver function

Lung

Time (hours)	% injected protein/ litre	Decay Correction Factor	Percent injected activity/ litre	Fractional injected activity/ litre	Monoexponential Estimate	Error measured value- modelled value
3.75	3.35	1.01	3.31	0.033	0.031	0.0002
25.12	3.02	1.09	2.76	0.028	0.024	0.0005
73.10	2.31	1.30	1.78	0.018	0.014	0.0009
142.45	1.82	1.67	1.09	0.011	0.006	0.0020
217.17	1.09	2.19	0.50	0.005	0.003	0.0011
5.50	3.40	1.02	3.33	0.033	0.030	0.0003
4.40	4.09	1.02	4.02	0.040	0.032	0.0023
26.30	3.69	1.10	3.35	0.034	0.024	0.0029
49.43	2.89	1.19	2.42	0.024	0.018	0.0015

Activity Decay parameters	
Half-life (hours)	60.2
Fractional injected activity/ litre @ t=0	0.032

Table A.5: Data spreadsheet for determination of population whole organ residence times.

Organ	Ao	alpha $t_{1/2}$	Bo	beta $t_{1/2}$	AUC	Mass of organ kg	Specific Density	Whole organ Residence time MBq.hrs	Whole organ Residence time MBq.sec
Lung	0.03	60.22			2.79	1.00	0.20	13.96	50265.70
Heart	0.08	64.63			7.19	0.32	1.00	2.27	8178.77
Liver	0.05	87.44			5.88	1.90	1.00	11.17	40198.73
Spleen	0.05	64.85			4.62	0.18	1.00	0.84	3040.92
Kidney	0.03	77.73			3.88	0.30	1.00	1.16	4178.56
Blood	0.04	9.22	0.14	51.02	10.63	5.50	1.00	58.46	210467.73

Outline of Spreadsheets used to determine activity biodistributions in normal tissues and tumour.

Table A.1

In this spreadsheet information regarding the times of scanning in relation to antibody administration is shown. In addition the SPECT correction factor used to convert counts per voxel into activity per ml is shown along with the scanning acquisition times.

Table A.2

In this spreadsheet data from region of interest delineation are entered. Regions of interest are placed in triplicate on areas of normal organs and tumour for each scan. For each ROI the slice, whether the ROI was drawn on the abdominal or thorax scan, the number of pixels and the total counts in the ROI are entered.

difference is summed at the foot of the table and the Excel solver function used to minimise this difference by adjusting the values of A_0 and $T_{1/2}$ in the activity decay parameter box. In such a way a mono-exponential or bi-exponential decay model was produced to describe the activity distribution in normal tissues

Table A.5

In this spreadsheet whole organ residence times are generated. Following mono and bi-exponential modelling, an estimate of the area under the curve is made per litre of tissue by direct integration using the equation $A_0 / (\ln(2)/t_{1/2})$. For tumours, where activity biodistributions frequently did not follow a mono-exponential decay pattern with a period of accumulation followed by variable retention and then falling activity, the area under the curve was estimated using the trapezoidal method. For points beyond the observation period in tumour, terminal decay parameters were assumed to be those of the blood clearance parameters calculated in the corresponding patient as modelled above. Using Olinda values of population averages of organ mass along with their specific density (assumed to be 1 for all organs except lung, which is given a specific density of 0.2) whole organ and tumour residence time in MBq.hrs and MBq.sec are generated. This data was subsequently entered into Olinda software along with the rest of body contribution. The latter was obtained by removing organ decay data at each time point from the whole body data and then subsequently constructing a monoexponential decay model using Excel solver in a similar fashion to above in order to allow a calculation of the rest of body contribution to be made.

In such a way for the study population an estimate of the normal organ and tumour absorbed doses as mGy per MBq were generated.

Appendix 2: Preliminary investigation of the feasibility of using tracer doses of ^{131}I -CHT25 and whole body clearance to allow for individual patient dosing using the method of Wahl et al (378)

In the technique of Wahl et al the administered activity of ^{131}I conjugated with tositumomab for therapy is individualised based upon the whole body clearance of a tracer labelled dose of antibody. In such a way treatment is administered with the aim of delivering a whole body dose of 75 cGy. Whole body dose is used as a surrogate for marrow absorbed dose and this level has been shown to be associated with predictable and acceptable levels of haematological toxicity. In order to administer therapy in this way a number of measurements are required including the patients height and mass, with the latter corrected to give the maximum effective mass and in addition an estimate of the whole body clearance times.

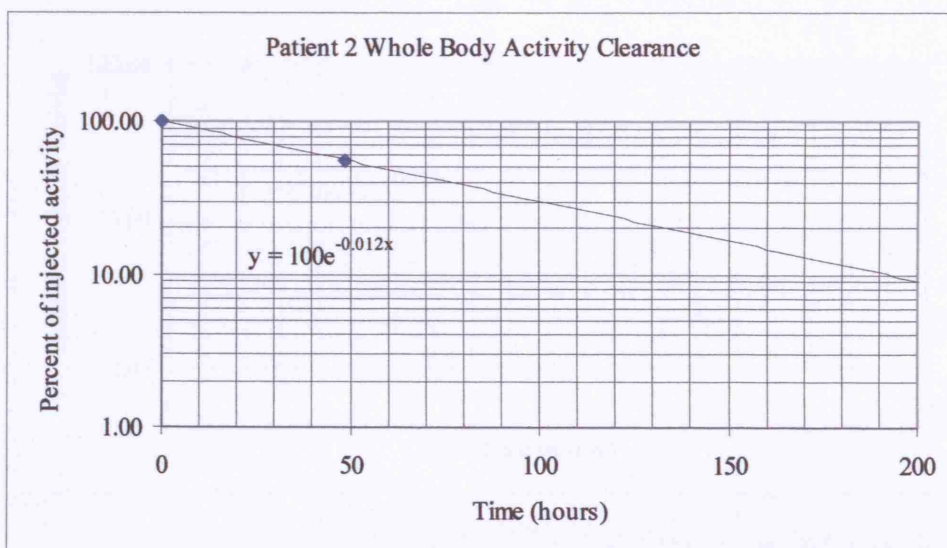
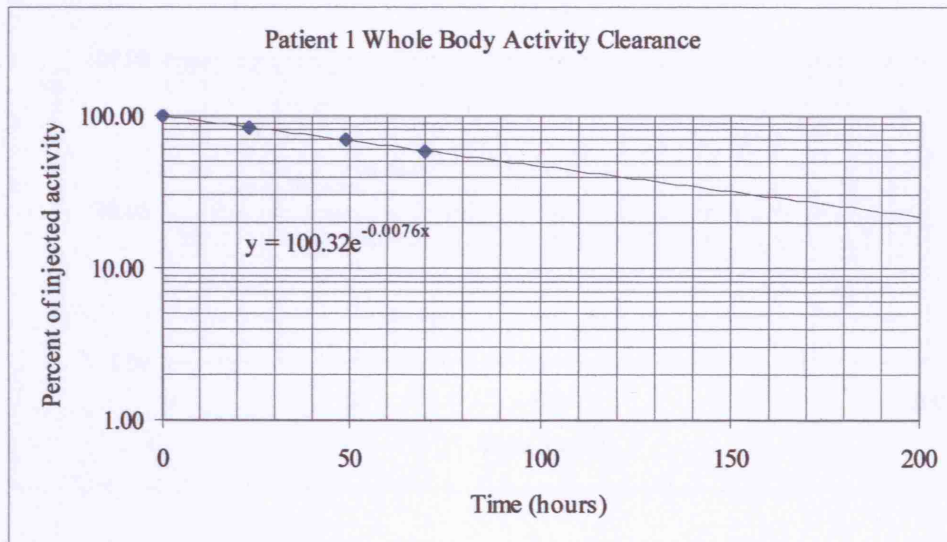
Although treatment with ^{131}I -CHT25 is not currently administered in this way, all the relevant information that is required for these calculations is available from the therapy doses already administered to allow an estimate of total body absorbed dose in individual patients. I have therefore applied this technique in order to further quantify the effects of altered biodistribution upon the likely toxicity following therapy.

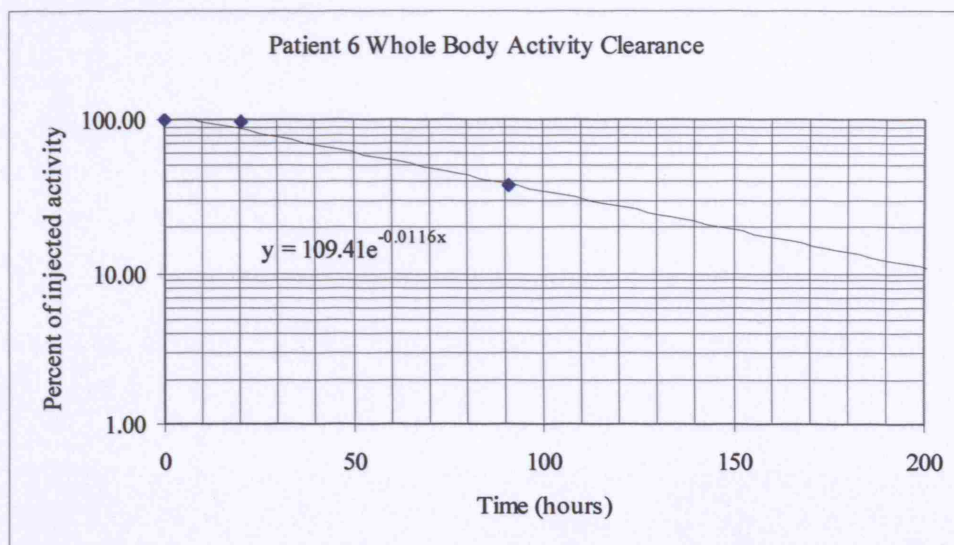
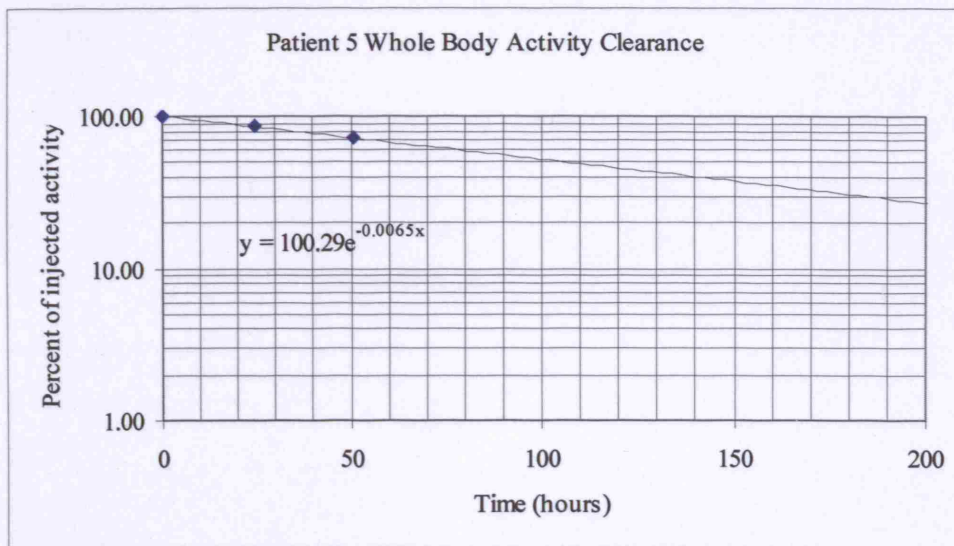
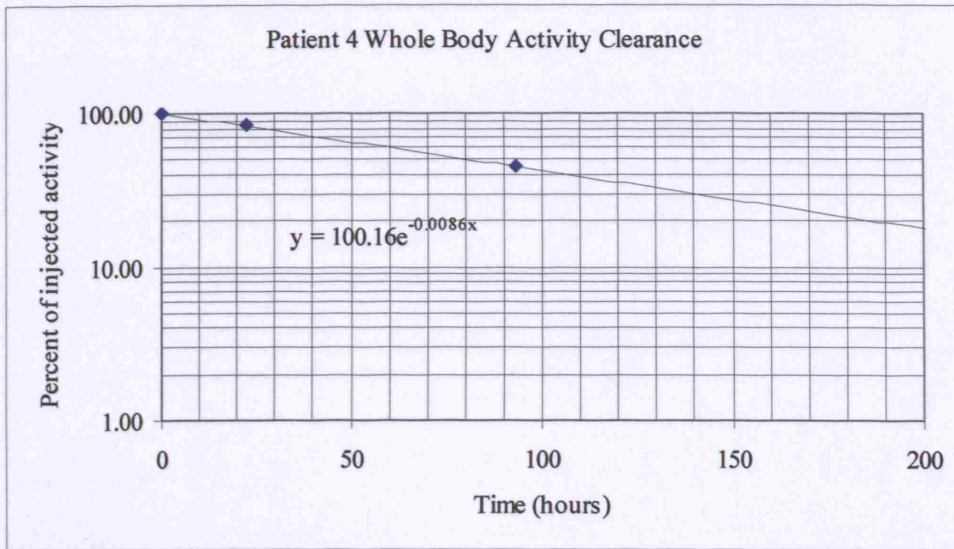
Method

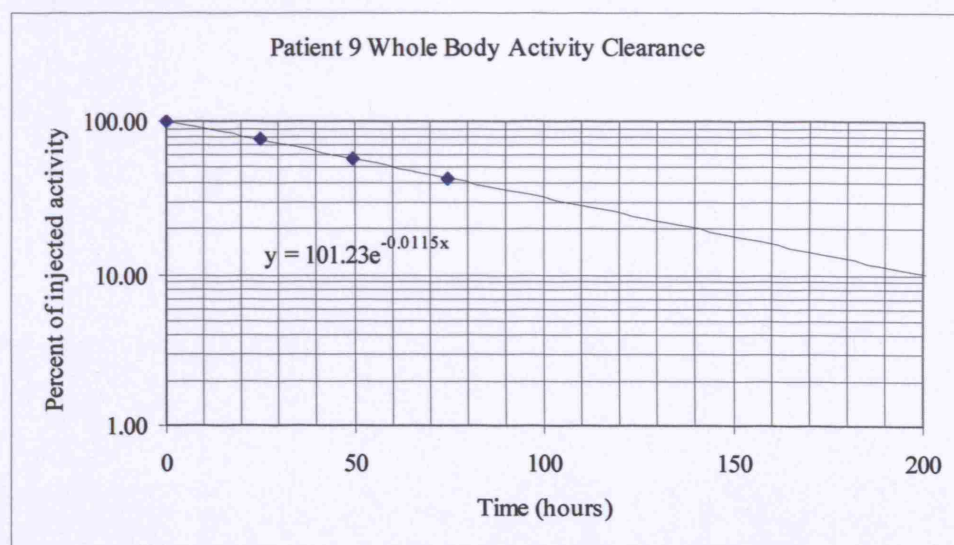
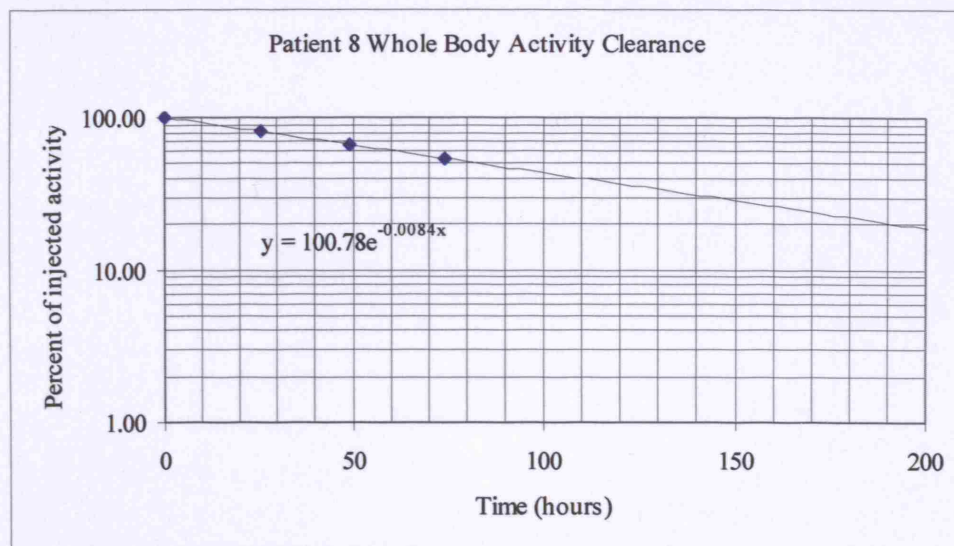
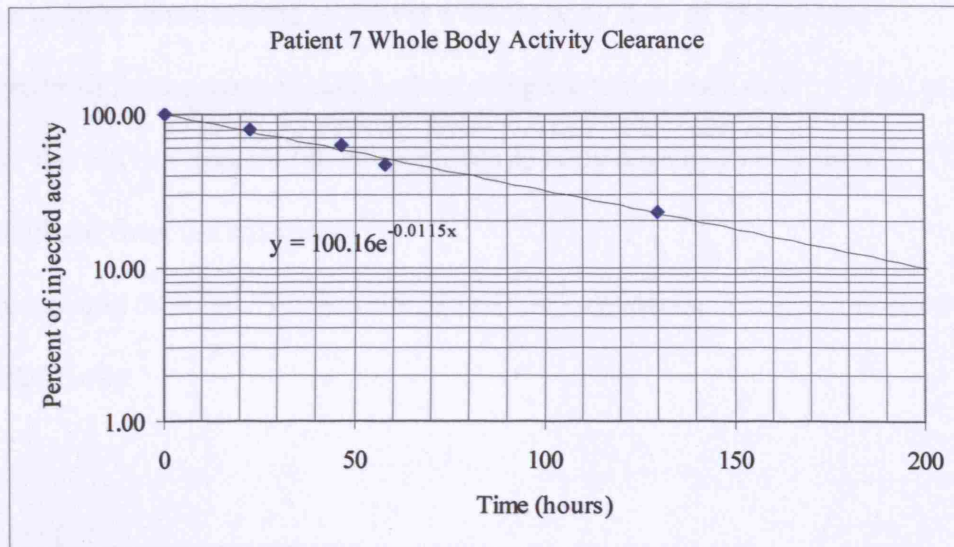
The method for calculating whole body dose is fully described in Wahl et al (378)

Whole body clearance estimates following initial exposure to ^{131}I -CHT25 was available for 8 of the 9 patients and estimated using measurements from a hand held dosimeter taken at fixed geometry. Whole body radioactivity was assumed to represent 100% of injected activity at time zero and along with subsequent values plotted on a semi-logarithmic scale. A linear plot was then fitted using excel and the residence time (in hours) was read from the X-axis of the graph at the point where this line intersected 37% of injected activity.

Figure B1: Whole body residence times estimated for 8 patients following initial therapy with ^{131}I -CHT25.







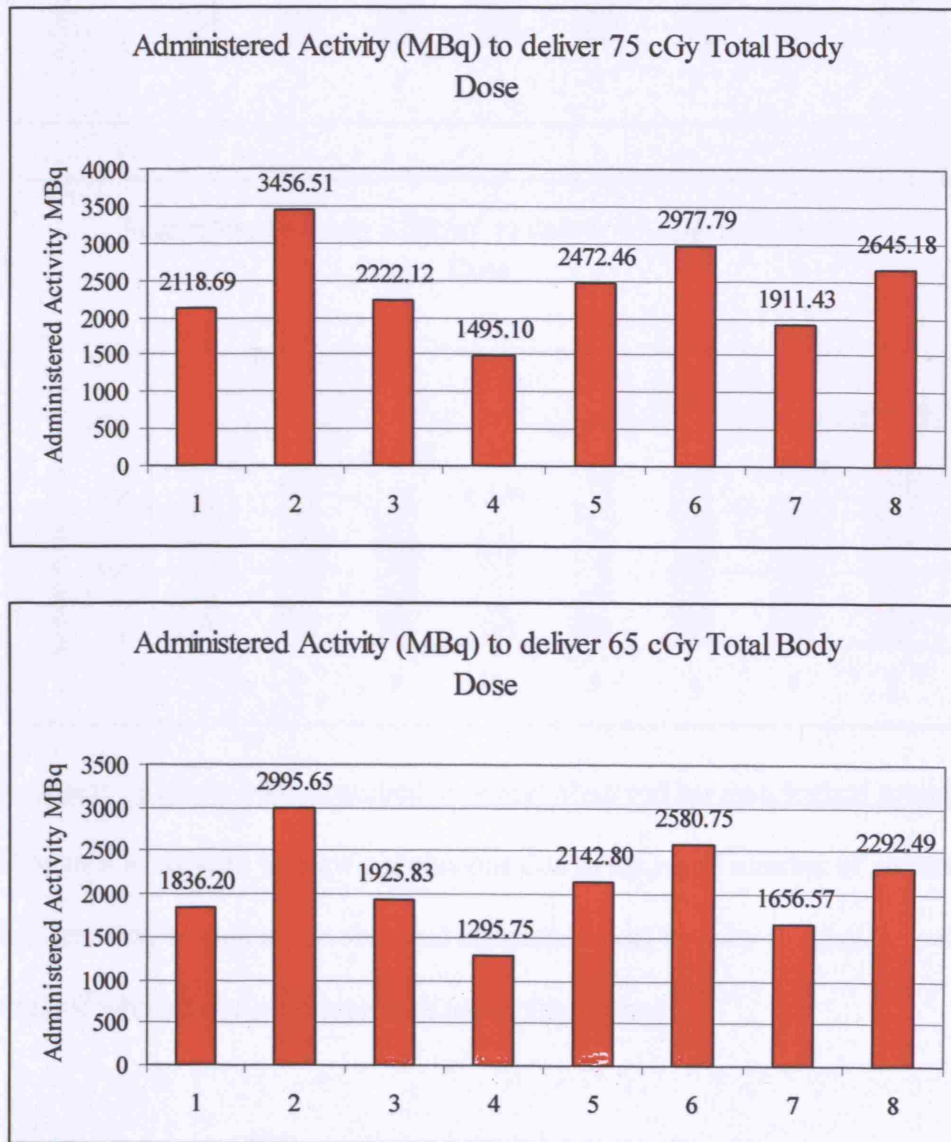
The activity hours needed to deliver a whole body dose of 75c Gy was calculated for the mass of each patient using the tables published by Wahl et al and the activity required to deliver a whole body dose of 75 cGy then calculated from the equation:-

$$\text{Therapeutic dose (mCi)} = \text{Activity hr (mCi.hr)} / \text{residence time (hr)} \times \text{desired TBD/75cGy}$$

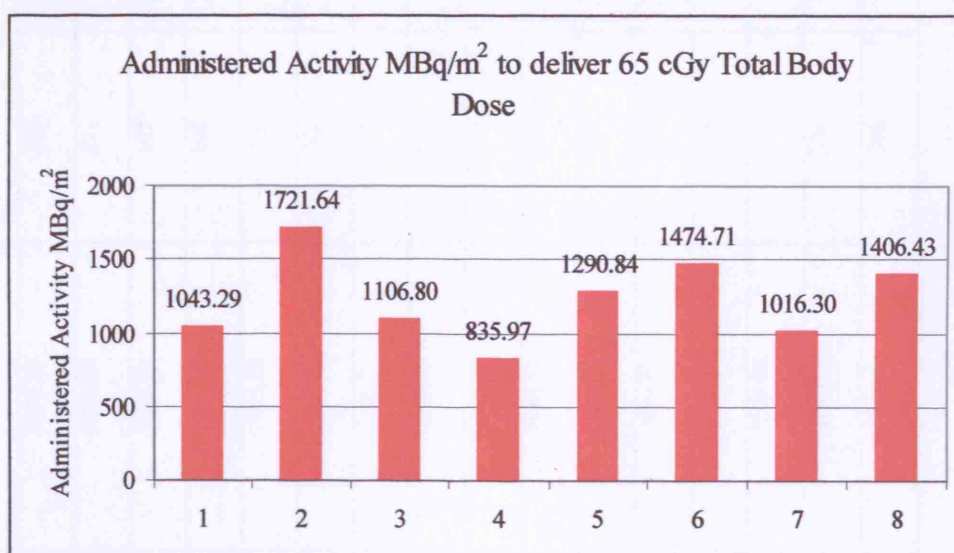
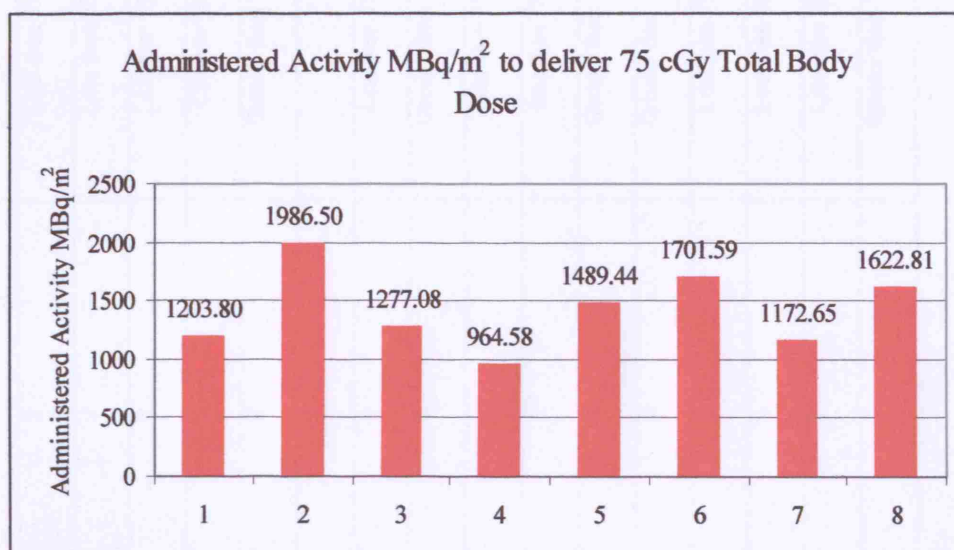
Results

Using this technique the administered activity required delivering a whole body dose of 75 cGy or 65 cGy varied considerable between patients as is shown in figure B2 below.

Figure B2: Administered activity required to deliver a whole body dose of 75 or 65 cGy



In order to correlate more easily these results with the data available from the clinical trial, this data is also presented as administered activity/m² to deliver a whole body dose of 75 cGy and 65 cGy is shown in figure 3.



Correlation of whole body absorbed dose and observed haematological toxicity

Although it is difficult to draw conclusions due to the small number of patients it is interesting to look at the observed haematological toxicity in relation to the estimated whole body dose estimates using this method.

Patient number and therapy number	Administered activity (MBq)	Administered activity to deliver 75c Gy TBD	Administered activity to deliver 65c Gy TBD	Platelet count at prior to therapy	Maximal CTC grade of haematological toxicity (excluding lymphocytes)	Comment
1	663	2118.69	1836.20	200	2	Less than 75 cGy
2	573	3456.51	2995.65	221	2	Less than 75 cGy
4 # 1	1380	2222.12	1925.83	185	2	Less than 75 cGy
4 # 2	1868	2222.12	1925.83	108	3	Close to 75 cGy
4 # 3	3395	2222.12	1925.83	24	4	Greater than 75 cGy
4 # 4	554	2222.12	1925.83	32	3	
5 #1	1286	1495.1	1295.75	179	2	Less than 75 cGy
5#2	2220	1495.1	1295.75	114	4	Greater than 75 cGy
6	1104	2472.46	2142.80	813	2	Less than 75 cGy
7#1	2395	2977.79	2580.75	274	3	less than 75 cGy
7#2	3560	2977.79	2580.75	87	4	Greater than 75 cGy
7#3	4553	2977.79	2580.75	46	4	Greater than 75 cGy
8	1093	1911.43	1656.75	401	2	Less than 75 cGy
9#1	1105	2645.18	2292.49	846	2	Less than 75 cGy
9#2	2104	2645.18	2292.49	384	1	Less than 75 cGy
9#3	3239	2645.18	2292.49	266	3	Greater than 75 cGy

Discussion

In patients with CD20 expressing NHL, administering the ^{131}I -tositumomab using the technique described by Wahl et al has resulted in reproducible and manageable haematological toxicity. Furthermore detailed bone marrow dosimetry performed by Boucek et al following the use of ^{131}I Rituximab has confirmed the correlation between whole body absorbed dose and bone marrow dose using such an approach (386). I have therefore applied the same technique to estimate the administered activity that would need to be administered in patients treated with ^{131}I -CHT25 study using information from the first administration of antibody as a 'tracer dose'.

The results confirm that due to altered biodistribution between patients, the administered activity that would be predicted to deliver a constant 75 cGy total body dose varied considerably from 1495 MBq to 3456 MBq. Interestingly looking at the actual activities delivered to the patients during the trial, all of the patients receiving an administered activity predicted to deliver a whole body dose of greater than 75cGy experienced either grade 3 or grade 4 (non-lymphocyte) haematological toxicity. Moreover, only 20% of the study patients receiving less than 75cGy whole body dose experienced grade 3 or 4 haematological toxicity. Of these 2 treatments one was close to the 65cGy total body dose that would have been administered using the Wahl regimen (patient 4#2 platelets 108 at baseline, administered activity 1868 MBq, 75 cGy whole body dose predicted administered activity 1925 MBq). The latter cases may reflect the more heavily pre-treated nature of the current study population and in particular the fact both these patients had previously undergone high dose procedures with stem cell rescue. It is also interesting that from the dose

escalation study currently it is anticipated that the non-myeloablative MTD lies between 740 and 1480 MBq/m². In this analysis the range of activities to deliver a 75 cGy whole body dose ranged between 965 MBq/m² and 1986 MBq.

Conclusions

It is difficult to draw firm conclusions from such a small data set, but this analysis suggests that a whole body dose of 75 cGy, or maybe 65cGy given the more heavily pre-treated nature of the study population, delivered upon the basis of whole body clearance, may be an appropriate administration schedule worthy of further investigation. Additional data collected from further patients undergoing therapy in the current protocol would allow a more robust verification of these results.

Appendix 3: Literature update

Introduction

The use of antibodies radiolabelled with beta emitting radionuclides has proved an effective therapy for the lymphomas and leukaemias and ongoing research is concerned with defining the exact role for these agents in the clinic. In RIT of solid tumours where therapy has seen less success the search for methods to augment the efficacy of therapy are currently being sought. Improved radiation dosimetry leading to the improved delivery of radioimmunotherapy is also being developed and is hoped will improved the therapeutic ratio of all radioimmunotherapy. In this section I shall review recent developments in RIT with particular reference to the work presented in this thesis.

Radioimmunotherapy targeting CD20 in the non-myeloablative setting

⁹⁰Y Ibritumomab tiuxetan was approved by the FDA in February 2002 and was the first RIT approved for use in the United States. More recent studies looking at its use in relapsed low grade and transformed NHL have confirmed its single agent activity in this setting (447;448). Given the success of RIT in this setting, Press et al have investigated the combination of ¹³¹I-Tositumomab in combination with CHOP in 90 patients with previously untreated advanced stage follicular lymphoma (449). In this study the overall response rate was 91% with a 69% complete response rate. After a median follow up of 5.1 years, the estimated 5 year overall survival rate was 87% with a progression free survival of 67%. These results were significantly better than those of historical controls treated with Rituximab with CHOP and a phase III randomised study is currently underway to compare the two regimes.

Initial approval of ^{90}Y Ibritumomab tiuxetan was limited to patients with adequate bone marrow reserves, defined as greater than 15% cellularity, less than 25% marrow involvement and no history of myeloablative therapy. Jacobs et al have looked at 8 patients with CD20 positive NHL who had previously received myeloablative chemotherapy, but not total body irradiation, as part of their conditioning and administered full dose therapy with ^{90}Y ibritumomab tiuxetan (450). Although the tumour response rates were limited (1/7), this is perhaps not surprising given the heavily pre-treated nature of the study population, only a single episode of neutropenic fever was noted and all patients were off blood product support 12 weeks following therapy. This data suggests that RIT used in this setting is no more toxic than when given to patients with relapsed or refractory NHL in whom previous myeloablative therapy has not been given (451).

In another study ^{131}I -Rituximab was administered to nine patients with relapsed, refractory or transformed NHL (452). In this study 4 of the patients had undergone previous high dose procedures with autologous stem cell transplantation. An administered activity to deliver a 45 cGy whole body dose was administered (2,200 \pm 600 MBq) was administered. In this study grade 3 or 4 haematological toxicity was seen in 7/9 patients with an increasing incidence in patients who had received more than two previous chemotherapy regimes. However this relatively high incidence of cytopenias was not associated with any significant clinical problems. Of the ten treatments administered to 9 patients, responses were seen in 4 with a single complete

response (452). It is interesting to compare this data with that of Turner et al who administered ^{131}I -Rituximab to patients with relapsed B-cell NHL and in whom a whole body dose of 75 cGy was administered (453). In this study an overall response rate of 76% was seen with a complete response rate of 59%. Grade 4 neutropenia was seen in 16% of patients. In this group of patients, although heavily pre-treated, no patient had previously undergone a high dose procedure.

This data suggests a potential role for radioimmunotherapy even in patients who have undergone a high dose procedure, although there is a suggestion of increased haematological toxicity. This data is concordant with the data presented in this thesis, where acceptable haematological toxicity has been observed in a heavily pre-treated group of patients, most of who had previously undergone a high dose procedure. In addition re-treatment with ^{131}I -Tositumomab has been shown to be safe in patients with low grade, follicular or transformed low grade B-cell NHL in whom a previous response to ^{131}I -Tositumomab had been seen (454). In this study of 28 patients responses were seen in 56% of patients with a 25% complete response rate and haematological toxicity was similar to that observed following initial therapy.

Radioimmunotherapy using CD20 targeting antibodies as Conditioning for high dose chemotherapy

The use of RIT in association with autologous stem cell transplantation for the treatment of B-cell lymphomas is currently an area of great interest. On first principles RIT is an attractive treatment to combine with high dose procedures

given the high response rates seen with ^{90}Y Ibritumomab tiuxetan and ^{131}I Tositumomab and also the lack of non haematological toxicity seen with their use. Following their initial use as single agents in relapsed disease and then at higher doses with stem cell support, these agents have now been combined with high dose chemotherapy prior to autologous stem cell transplantation. Their use in this setting has been either to replace total body irradiation (TBI) or to augment standard chemotherapy regimes. Results so far are promising with combinations of radioimmunoconjugates and chemotherapy producing long lasting responses in high-risk patients with no more toxicity than that caused by standard conditioning regimes.

Following on from their initial work using ^{131}I labelled antibody prior to autologous stem cell transplantation, Press et al have combined RIT with high dose chemotherapy in patients hoping to deliver higher tumour doses of radiation than is possible with TBI. In a phase I/II study looking at 52 patients with relapsed/ refractory NHL combining ^{131}I -Tositumomab with high dose etoposide and cyclophosphamide toxicity was no different from historical patients receiving TBI and the same chemotherapy. In addition comparison to this non randomised control group of patients suggested a better overall survival and progression free survival in the RIT group after multivariate analysis accounting for differences in the patient group characteristics (455). The same group has also adopted a similar approach in patients with mantle cell lymphomas; a disease characteristically resistant to standard therapy and commonly treated with high dose therapy, followed by autologous or allogeneic stem cell transplant (456). In this study response rates were high with all patients

responding and 91% achieving a complete remission with estimated 3-year overall and progression free survivals of 93% and 61% respectively. The toxicity profile was similar to that of standard autologous transplant regimens (457). Although no phase III randomised data are yet available comparing RIT and high dose chemotherapy with conventional high dose therapy approaches, Gopal et al have compared outcomes of 27 patients with follicular lymphoma enrolled in phase I and II trials of high dose chemotherapy plus ¹³¹I-Tositumomab with outcomes of 98 historical controls (458). After multivariate analysis for confounding factors in the patient populations, those receiving RIT had an improved overall and progression free survival (67% vs. 53% and 48% vs. 29% respectively).

Initial studies using dose escalated ⁹⁰Y ibritumomab tiuxetan in the transplant setting combined increasing doses of radioimmunotherapy with either BEAM chemotherapy or etoposide and cyclophosphamide. In a phase I study reported by Winter et al individualised dosimetry was used to provide a cohort defined radiation dose to critical organs. Although toxicity was similar to that seen in patients treated with BEAM alone, patient absorbed radiation doses to critical organs were variable, suggesting that dosing based upon weight and not dosimetry would be likely to result in a wide range of absorbed doses to critical organs (459). Data has also been published from a phase I/II study combining high dose ⁹⁰Y Ibritumomab tiuxetan with high dose etoposide in 31 patients with follicular, diffuse large B-cell and mantle cell lymphoma (460). At a median follow up of 22 months, the 2-year estimated overall survival was 92% and the disease free survival was 78%. In this study toxicity was similar to those seen

following standard transplant regimes. These results are similar to those reported by Press et al using ^{131}I -Tositumomab and the same doses of etoposide and cyclophosphamide (455).

Autologous stem cell transplantation has an important therapeutic role in patients with aggressive or refractory NHL. Pre-treatment conditioning regimes composed of a combination of total body irradiation and high dose chemotherapy may be the most efficacious but also the most toxic.

Radioimmunoconjugates have the capacity to deliver higher doses to the tumour site than to normal tissues without increasing treatment related toxicity and in all of the studies cited above, no increase in treatment related toxicity has been reported in comparison to conventional high dose chemotherapy procedures.

Furthermore the dose of radiation delivered to tumour is 10-fold higher than that possible with TBI (461). With regard to toxicity the possibility of an increase in the incidence of secondary acute myeloid leukaemia (AML) and myelodysplasia (MDS) associated with RIT and autologous stem cell therapy has been suggested. However a recent review of over 1000 patients treated with ^{131}I -Tositumomab has shown that the incidence of AML and MDS was no different from that expected for patients heavily pre-treated with other chemotherapy for NHL (462).

CD20 radioimmunotherapy using α emitting radionuclides

Single cell and small cancer cell clusters may be ideally suited to therapy with α emitting radionuclides given their high LET and shorter range. Rituximab has been radiolabelled with ^{211}At (463) and ^{149}Tb (464). Aurlen et al have

demonstrated cell specific cytotoxicity in pre-clinical studies using ^{211}At , but clinical applications using this nuclide is limited due to its short half life and the need for cyclotron production (463). Beyer et al have radiolabelled rituximab with ^{149}Tb and demonstrated evidence of efficacy in a SCID mouse model of leukaemia (464). ^{149}Tb is a partial alpha emitting radionuclide with an effective half life of 4 hours with chemical behaviour similar to ^{90}Y and ^{177}Lu allowing conjugation to antibody using existing approaches. Advantages of ^{149}Tb over ^{213}Bi include a lower energy and higher LET of emitted particles and a longer half life; these features simplify preparation of the bioconjugate and likelihood of achieving adequate tumour localisation following administration although the activity of long lived daughter radionuclides also needs to be considered. In this model system therapy with radiolabelled activity was associated with prolonged disease free survival in contrast to the control groups, which received unlabelled rituximab.

CD25 targeting RIT

Paik et al have recently reported in abstract form the results of a phase I/II dose escalation study looking at the use of ^{90}Y labelled anti-tac antibody in patients with CD25 expressing malignancies (465). In an ongoing study 23 patients with a variety of CD25 expressing malignancies have been treated. Admission criteria included expression of CD25 on at least 10% of tumour cells, platelet count greater than $100,000/\text{mm}^3$ with all patients being heavily pre-treated. Patients were treated at 6 weekly intervals until disease progression, DLT or completion of seven cycles. The single administered activity MTD was 555 MBq although 2 patients with Hodgkin's disease tolerated cumulative activities

of 2.2 of 3.3 GBq without myelosuppression. Objective responses have only been seen in patients with Hodgkin's disease with 2 complete responses, 2 partial responses and 4 stable diseases out of a total of 10 patients. The median survival of all patients was 5 months. Humanised anti-tac antibody has also been radiolabelled with a number of α emitting radionuclides including ^{212}Bi , ^{211}At (466) and ^{213}Bi (467). Wesley et al have investigated the use of humanized anti-Tac armed with alpha-emitting radionuclides ^{212}Bi and ^{211}At in a cynomolgus monkey cardiac allograft model. Control graft survival was not significantly different for animals receiving ^{212}Bi anti-tac in comparison to animals receiving anti-tac alone, a fact though to relate to the short half-life of the isotope. However a significant improvement in survival was seen over naked anti-tac with the ^{211}At labelled antibody. The authors conclude that this agent may have application in organ transplantation and in the treatment of IL-2 receptor expressing T-cell leukaemia. The efficacy of ^{213}Bi labelled anti-tac has also been investigated in a murine model of human adult T-cell leukaemia using a pre-targeting approach (468). Pre targeted ^{213}Bi anti-tac was more effective than non pre-targeted ^{213}Bi anti-tac. Furthermore there was no significant therapeutic efficacy when using pre-targeted ^{90}Y anti-tac. The best results were seen using pre targeted ^{213}Bi anti-tac in combination with 4 weekly doses of naked anti-tac.

Fractionated RIT

Further pre-clinical work has been published in support of fractionation of RIT in a gastric cancer tumour model. In this study locoregional radioimmunotherapy of intraperitoneal disseminated gastric cancer was studied using ^{213}Bi labelled antibody d9MAb with the prime aim of comparing the

therapeutic efficacy and toxicity of single versus double injections of antibody. In this nude mice fractionated therapy was associated with improved therapeutic effect in both early and advanced stage disease in comparison to a single injection of equivalent activity (328). Double application of only 0.37 MBq of ^{213}Bi -d9MAb at days 1 and 8 after tumour cell inoculation significantly prolonged median survival in nude mice compared with a single injection. Even in an advanced stage of the disease, double injection of 0.74 MBq at days 8 and 15 was superior to a single injection of 1.48 MBq at day 8. However in a clinical study looking at fractionated therapy using ^{131}I labelled chimeric antibody G250 in patients with metastatic cancer, no significant reduction in marrow toxicity or improvement in maximum tolerated activity was observed by fractionating therapy at 2-3 day intervals. In this instance a maximum activity that delivered 75 cGy whole body dose has been achieved which is not significantly different from that observed with other ^{131}I labelled antibodies administered as a single fraction (469). In this study however pharmacokinetic studies including whole body pharmacokinetics and serum clearance pharmacokinetics accurately predicted the clearance of subsequent administrations.

Combined approaches

In a pre-clinical model ^{90}Y -DOTA-cPAM4 antibody has been given in combination with Gemcitabine in a murine model of human pancreatic cancer. In this study Gemcitabine administered as a single agent provided no antitumour effect whereas a single cycle of radiolabelled antibody and Gemcitabine provided effective tumour control. The authors suggest that the addition of ^{90}Y -DOTA-cPAM4 to Gemcitabine may provide enhanced efficacy the treatment of

pancreatic cancer (470). In another pre-clinical breast cancer tumour model concomitant Paclitaxel has been shown to enhance the therapeutic effect of RIT, potentially because of its ability to arrest cells in the radiosensitive G(2)/M phase of the cell cycle (471).

Single-chain Fv (scFv) antibody fragments exhibit enhanced and more rapid tumour uptake than intact IgG and clear more rapidly from the blood. However, they also exhibit lower net dose deposition within tumour due to a shorter overall residence time that limits their use in radioimmunotherapy. To improve the tumour uptake and retention of scFv, Jain et al have investigated the utility of the cell-penetrating peptides, penetratin and transactivator of transcription (TAT) in a murine colorectal xenograft model. In their study Penetratin and TAT increased the tumour retention of scFv without affecting the peak dose accumulation and also improved the tumour-to-blood ratios. Furthermore autoradiography of excised tumours indicated a more homogenous distribution of the radiolabelled scFv with both penetratin and TAT in comparison with the control treatment. These results suggest that the combination of penetratin and scFv has the potential of improving the effectiveness of RIT using scFv fragments (472).

Another approach that has been used to improve the accumulation of radiolabelled antibodies in tumour has been the use of STI571 (Gleevec), a platelet derived growth factor receptor β antagonist, which has been shown to reduce the interstitial pressure in tumours. In a murine model the combination reduced tumour interstitial fluid pressure by more than 50% and in so doing

improved both the absolute uptake of B72.3 and the homogeneity of antibody distribution. These effects were dose-dependent and under optimized dosing conditions allowed for a 2.45 times increase in the tumour uptake of B72.3. The increased uptake of radioimmunotherapy into tumour resulted in a greater than 400% increase in the tumour absorbed radiation doses in STI571 + radioimmunotherapy-treated mice compared to controls treated with PBS and radioimmunotherapy. An additional factor that was noted which may also have a significant role in improving the efficacy of RIT was an increase in tumour radiosensitivity resulting from improved tumour oxygenation (473).

Radioimmunotherapy in microscopic disease setting

A recent study has investigated the use of RIT in patients with colorectal cancer following salvage resection of liver metastasis (474). In a phase II study twenty-three patients who underwent surgery for liver metastasis of colorectal cancer received a dose of 40 to 60 mCi/m² of ¹³¹I-labetuzumab, a humanized monoclonal antibody against carcinoembryonic antigen. With a median follow-up of 64 months, the median overall survival time from the first liver resection was 68.0 months, the median disease free survival time was 18.0 months and the 5-year survival rate was 51.3%. The major adverse effect was transient myelosuppression, which was manageable and reversible. In comparison to historical controls treated with adjuvant chemotherapy following resection in the same institution both the median overall and 5-year survival rates seem to be improved with adjuvant radioimmunotherapy (474). The authors concluded that these results justify further evaluation in a multicentre, randomized clinical trial. In another study looking at ¹³¹I-huA33, a humanised monoclonal antibody

targeting the A33 antigen expressed on 95% of colorectal cancer excellent tumour targeting has been seen, but only limited efficacy in 15 patients with pre-treated metastatic cancer. The authors concluded that a combined approach with chemotherapy to enhance efficacy was probably necessary to achieve therapeutic effect in this patient population (475).

Radiation Dosimetry

The use of whole body dose to determine administered activity is now routinely practiced in the administration of ^{131}I -tositumomab and is being increasingly used in the administration of other radioiodinated antibodies. A novel technique of quantitation of bone marrow uptake using hybrid SPECT/CT imaging was developed by Boucek et al to validate this methodology using post-RIT extended imaging and data collection. In patients treated with ^{131}I -rituximab a strong, statistically significant correlation between whole-body effective half-life of antibody and effective marrow half-life was demonstrated (386).

The advent of CT-SPECT imaging allows for activity distributions to be correlated with detailed anatomical data overcoming the limitations imposed by the poor spatial resolution of SPECT (476). However because of more accurate scatter and attenuation corrections and improved spatial and temporal resolution, PET has been suggested as a better imaging tool for performing tracer studies to guide therapy in RIT (477). However due to their short half-lives, the commonly used PET emitting radionuclides, such as $^{94\text{m}}\text{Tc}$, ^{68}Ga and ^{18}F , would only appear suitable for studies looking at small antibody fragments which clear rapidly. However the isotopes ^{124}I and ^{89}Zr , with half lives of 78.4 and 100.3

hours respectively, might be appropriate for performing tracer studies for future therapy with ^{131}I given their similar half lives. However clinical experience with immuno-PET remains limited due to a lack of robust labelling procedures and availability of suitable positron emitters. Moreover little pre-clinical evidence has been provided that immuno-PET can be used as a reliable tracer tool in radioimmunotherapy. However recently Verel et al have provided compelling evidence that the use of long lived PET agents in this setting is worthy of further investigation (478;479). The major requirement for the use of immuno-PET as a tracer procedure is that the biodistributions of the diagnostic and therapeutic radioimmunoconjugates are similar. In a murine squamous cell carcinoma xenograft model, the positron emitters ^{89}Zr and ^{124}I were used to label the head and neck squamous cell carcinoma selective chimeric antibody U36. The antibody was also labelled with the therapeutic radionuclides ^{88}Y , ^{131}I , and ^{186}Re . In this study the biodistribution of ^{89}Zr matched that of ^{89}Y and ^{124}I matched closely that of ^{131}I and ^{186}Re suggesting a potential role for tracer studies using these therapeutic isotopes (with ^{88}Y being a surrogate for ^{90}Y) (479). In a subsequent study labelled U36 was labelled ^{124}I using an improved chelation method and was again demonstrated in a pre-clinical model to yield similar biodistribution to the same antibody labelled with ^{131}I further supporting its use as a potential tracer isotope for immuno-PET (478).

In order to convert activity distribution maps into absorbed dose maps a number of groups are using a variety of dosimetry methods including voxel based MIRD techniques (480) and dose kernel convolutional methods (481;482). Monte-Carlo based algorithms give most accurate results and use SPECT or PET

activity arrays in combination with CT arrays of tissue density to simulate radioactive particle transport and score energy deposition within the body (483-487). Furhang et al have developed a Monte-Carlo based technique and validated the system by creating CT and SPECT arrays for the Medical Internal Radionuclide Dose (MIRD) Committee's Standard Man phantom, and reproducing the spatially averaged specific absorbed fractions reported in MIRD Pamphlet 5 to good effect (484;488). However the production of absorbed dose distributions across tissues demonstrated that hot and cold spots were common with typical variations of absorbed dose varying by a factor of 4 for small organs and a factor of 45 for large organs illustrating the significant limitations of the MIRD schema which assumes homogeneous activity distribution within organs.. In comparison to dose kernel convolutional methods in which tissue heterogeneities cannot be accounted for, it was demonstrated that although simulations were appropriate for photon emissions above 100 keV, results were less than satisfactory for radionuclides with initial energies less than 100 keV and highlighted the potential limitations of dose kernel convolutional methods (484;488).

With the increasing use of targeted radionuclide therapy an important consideration is the effect of co-administered external beam radiotherapy. Bodey et al have developed a system using the biologically effective dose in order to determine the contributions of both treatment modalities given that tissue effects will not be adequately understood using absorbed dose alone given the differing patterns of radiation delivery (489).

Conclusions

Radioimmunotherapy is an active area of ongoing research in both pre-clinical and clinical settings. Given the efficacy of CD20 targeting antibodies clinical research is broadening their application to use in a combined setting with systemic chemotherapy and as a replacement of TBI in the high dose setting. Recent data suggests that re-treatment with RIT is possible and may not be associated with any increase in marrow toxicity. This suggests that significant marrow recovery is possible and supports what is known of the radiobiology of marrow stem cells. This data is further support for the development of humanised antibodies for clinical use, which allow for repeated therapies to be given with a reduced likelihood of neutralising antibody formation.

The publication of Paik et al regarding the use of ^{90}Y labelled anti-tac and its effectiveness in patients with Hodgkin's disease is further supportive evidence for the continued development of ^{131}I CHT25 outlined in this thesis. With regard to fractionated RIT, a familiar pattern is seen in the recent literature presented here, namely that in the pre-clinical setting it seems to offer an advantage, whereas no real benefits are seen in the clinic. It is difficult to see how fractionation of a given activity of RIT will be beneficial using conventional radiobiological modelling, but other factors unique to RIT are also known to be important and although these did not appear significant in the work presented in this thesis, it is possible these vary depending upon the tumour type.

In an attempt to improve the therapeutic ratio of RIT in the common epithelial tumours there is ongoing interest in the use of combined approaches. Using

conventional chemotherapy encouraging improvements have been seen in pre-clinical models. A number of agents that improve the tumour delivery of RIT have also shown encouraging evidence of efficacy in pre-clinical models.

Radiation dosimetry continues to evolve and a number of different groups have developed validated individual dosimetry systems based upon CT-PET or CT-SPECT data sets and hopefully will become routinely used to guide RIT in the clinic, ultimately improving the safety and efficacy of RIT in the clinic. Finally a system demonstrating that combined effective dose estimates in patients receiving both external beam therapy and radioimmunotherapy is feasible is an important step in the safe and effective future integration of RIT into clinical treatment algorithms.

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